THE EFFECT OF MOULTING HORMONES ON THE GROWTH AND REPRODUCTION OF CERTAIN INSECTS

ABSTRACT

THESIS SUBMITTED FOR THE DEGREE OF

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BY

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ABSTRACT

In insects, ecydsones (moulting hormones) are naturally produced by the prothosacic glands of their larvae in the form of α- and β-isomers. The function of both the isomers is to bring about moulting during the growth. Apart from the natural source these ecdysones and their analogues have also been synthesized as well as isolated from plant sources (phytoecdysones).

Extensive work has been done by applying these hormones exogenously on the insects to observe their possible effect on growth and reproduction (Robbins et al., 1968 & 1970; Riddiford, 1970; Wright & Kaplanis, 1970; Earle et al., 1970; Thompson et al., 1971; Kaplanis et al., 1971; Wright et al., 1971; Walker & Thompson, 1973; Jalaja et al., 1976; Singh & Russell, 1980; Singh et al., 1982). Generally, these hormones had inhibitory effect on growth and reproduction and almost all workers reiterated the importance of these hormones for controlling the insects. However, some workers observed stimulating effects of these hormones, usually in sub-lethal doses, on the growth as well as reproduction of insects (Laverdure, 1969; Spielman et al., 1971; Chatani & Ohnishi, 1976; Herman & Barker, 1976; Went, 1978; Chudakova et al., 1982). However, Robbins et al. (1968) concluded that, in general, natural ecdysones were highly active in moulting whereas their analogues were potent

inhibitors of growth, metamorphosis and reproduction. But it can also be infered from the earlier data that there exists a vide variation in the susceptibility of different insects to these compounds and an equally pronounced difference in the response of a single species to different molecular structures. In view of this the present study was undertaken to investigate the effects of two natural ecdysones (α- and β-ecdysone) and an analogue ('Triol') on Spodoptera litura, Diacrisia obliqua and Dysdercus cingulatus to observe their effect on longevity, mortality and malformation in advanced growth stages (larval and pupal) as well as adult emergence, fecundity and fertility. All the three hormones were applied on individual insect and their doses were 0.5, 1.0, 2.0, 4.0 and 6.0 ug per larva/nymph.

The larvae (5th and 6th instar) of S. litura suffered mortality following the application of both β-ecdysone and 'Triol' on the larvae of respective instar by either injection or ingestion method. Although larval mortality occurred by the application of all the doses of these ecdysones, there was 100% larval mortality when 6.0 ug 'Triol' was injected to the 6th instar larvae of this species. Further, the application of exogenous β-ecdysone and 'Triol' also affected the larval longevity of this insect. The larval duration of 5th instar remained unchanged following the injection of any dose of β-ecdysone. But following the treatment of 6th instar larvae

the larval duration was shortened only by the strongest selected dose (6.0 ug) whereas lower doses were ineffective. On the other hand, the ingestion of the 6.0 ug dose of the same hormone by the larvae of both 5th and 6th instars caused abbreviation in their respective longevity. Application of the analogue 'Triol', either injection or feeding to the larvae of both 5th and 6th instars abbreviated the longevity of either instar much more as compared to that of β-ecdysone treatment. Further, when 6th instar larvae of S. litura were injected or fed with 6.0 ug β-ecdysone it resulted in the occasional formation of a supernumerary larval instar which was 16.66% by injection and 33.33% by feeding but treatment with 'Triol' did not produce such effect. Again, the injection of 6.0 ug dose of β-ecdysone to either 5th or 6th instar larvae of S. litura showed occasional occurrence of malformed pupae but injection of similar dose of 'Triol' did not produce such pupae. However, the ingestion of these hormones by the larvae of either instar showed higher incidence of malformed pupae. The number of adults formed was, thus, markedly reduced due to larval and pupal mortality following the application of both β-ecdysone as well as 'Triol' to the larvae of either instar of S. litura. In this respect injection of strongest dose (6.0 ug) of 'Triol' was most effective than that of β-ecdysone. Thus the number of adults emerging from 5th and 6th instar larvae injected with this dose of 'Triol' was reduced by 78% and 100% respectively.

However, the emerged adults were perfectly normal. It was also found that following the application of both β-ecdysone and 'Triol' to the advanced larval stages of S. litura, the fecundity and fertility of the emerged females was effectively inhibited. Maximum reduction in fecundity occurred in the females which emerged from 5th instar larvae injected with strongest dose of β-ecdysone (6.0 ug) i.e., out of 6 females only one female laid 76 eggs. Similarly, maximum reduction in fertility was observed in the females emerged from 5th instar (60.6%) and 6th instar (70.15%) larvae injected with 6.0 ug β-ecdysone.

In Diacrisia obliqua also, larval mortality was found when α-ecdysone as well as 'Triol' were individually injected or fed to the larvae of 5th or 6th instars. Maximum larval mortality (53%) was found in the 5th instar larvae ingesting 6.0 ug α-ecdysone. Further, the larval longevity was abbreviated when α-ecdysone was injected to 5th instar larvae but when this hormone was injected to 6th instar larvae, their longevity was unaffected. The longevity of both 5th and 6th instar larvae of D. obliqua remained unaffected following the ingestion of either dose of α-ecdysone as well as injection of 'Triol'. But ingestion of higher doses of this analogue (4.0 and 6.0 ug) abbreviated the larval longevity of either instar. Further, the treatment of the larvae by either α-ecdysone or 'Triol' produced malformed larvae represented as

neotenic forms, precociously moulted forms, larvae with partial ecdysis and also larval-pupal intermediates. Both the hormones when injected to 5th instar larvae of D. obliqua resulted in the frequent formation of malformed pupae. Such malformation was not found when 6th instar larvae were injected with any of these two hormones. Again, ingestion of α-ecdysone by either 5th or 6th instar larvae did not produce malformed pupae but ingestion of 4.0 ug and 6.0 ug doses of 'Triol' by either instar occasionally resulted in pupal malformation. Both the hormones lowered the formation of adults due to larval and pupal mortality. Maximum reduction (53%) in the number of adults was found when 5th instar larvae of D. obliqua were fed with 6.0 ug α-ecdysone. It was also recorded that adults emerged from the larvae treated with α-ecdysone as well as 'Triol' were occasionally malformed. Such adults had folded wings and maximum malformation (44.44%) appeared in the adults emerged from the 5th instar larvae injected with 6.0 ug dose.

There was marked decrease in both fecundity and fertility of the females emerged from the larvae treated with α-ecdysone as well as 'Triol'. Maximum reduction in fecundity (75.88%) was recorded in the females emerged from the 6th instar larvae injected with 6.0 ug dose of 'Triol'. But maximum reduction in the fertility (47.25%) was observed in the eggs of the females which emerged from 5th instar larvae fed with 6.0 ug 'Triol'.

In Dysdercus cingulatus also nymphal mortality was found by all doses following the treatment of both 4th and 5th instar nymphs with α-ecdysone as well as 'Triol'. Maximum nymphal mortality (64%) was found following the injection of 6.0 ug 'Triol' to 4th instar nymphs. The injection of α-ecdysone to 4th as well as 5th instar nymphs did not affect their nymphal longevity. The topical application of α-ecdysone also had no effect on the nymphal longevity of 4th instar whereas such application on the 5th instar nymphs adversely affected their longevity. Injection of 'Triol' to the 5th instar nymphs abbreviated the longevity of this instar but 4th instar nymphs were not affected by this analogue. However, topical application of all the present doses of 'Triol' affected the nymphal longevity of both the instars. There was no malformation in the nymphs following the treatment with both the hormones.

Adult emergence was also reduced due to nymphal mortality following the treatment of either instar with either hormone and maximum reduction (64%) was observed when 6.0 ug dose was injected to 4th instar nymphs. Both fecundity and fertility of the females emerged from the treated 4th as well as 5th instar nymphs were also adversely affected. Thus maximum reduction in fecundity (47.32%) was recorded in the females emerged from 4th instar nymphs topically treated with 6.0 ug 'Triol'. But maximum reduction in fertility (47.48%) occurred in the females emerged from 5th instar nymphs injected with 6.0 ug 'Triol'.

Although the present data on S. litura, D. obliqua and D. cingulatus are also variable with regard to the effect of the natural ecdysones and the analogue 'Triol', on the larval growth and reproductive physiology, it has been generally found that the application of the analogue is more effective in causing larval mortality, reducing adult emergence as well as egg production and their fertility. Therefore, for the control of these three species it can be suggested that 'Triol' may used in the concentration of 6.0 ug per larva on advanced larval stages of these pests, in comparison to the natural hormone. This can be preferred when chemical control of these pests is not desired due to toxic hazards.

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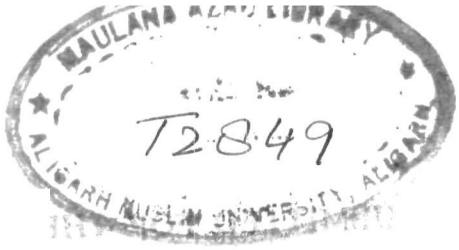
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Date December 30, 1983

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M.Sc.(Alig.), Ph.D.(Lond.), D.I.C., F.R.E.S.I
Reader in Zoology.

CERTIFICATE

This is to certify that Mr. Shakil Ahmad investigated on the research topic entitled, "The effect of moulting hormones on the growth and reproduction of certain insects" under my supervision. After careful assessment of the technical programme and data I permit him to submit his observations in the form of a thesis for the award of the degree of Doctor of Philosophy in Zoology, at Aligarh Muslim University, Aligarh, India.

Mumtaz A Khan

(MUMTAZ AHMAD KHAN).

C O N T E N T S

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Shakil Ahmad

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I. INTRODUCTION

In insects the moulting hormone (ecdysone) is secreted by the prothoracic glands (Karlson, 1963) and its secretion is principally regulated by ecdysiotrophin produced by the median neurosecretory cells of pars intercerebralis (Wigglesworth, 1973; Wyatt, 1971; Doan, 1973; Gilbert & King, 1973). It is well known that the larval growth and moulting of insects is induced by the coordination of hormones liberated from the corpora allata (juvenile hormone) and prothoracic glands (moulting hormones). Ecdysone provokes a developmental response when it accumulates to threshold titre at certain critical periods. Meanwhile, the titre of juvenile hormone dictates whether the developmental response will be moulting or metamorphosis.

The crystalline material of the moulting hormone was first isolated from Bombyx pupae and named ecdysone by Butenandt and Karlson (1954). It was further isolated and identified from adults of Moroccan locust, Dociostaurus maroccanus (Stamm, 1958); tobacco hornworm, Manduca sexta (Kaplanis et al., 1966b); oak silkworm, Antheraea pernyi (Horn et al., 1966) and blow fly, Calliphora stygia (Galbraith et al., 1969).

The moulting hormone extract from the insect source was shown to have two components (Karlson, 1956). The major compound was named α-ecdysone (or ecdysone) and the minor compound β-

ecdysone (20-hydroxyecdysone or 20-OH or ecdysterone). Several other ecdysteroids have been isolated from insects and ecdysone has been used as a generic term for insect moulting hormones. Goodwin et al. (1978) has proposed the use of ecdysteroids as the generic term with ecdysone and 20-OH ecdysone being reserved for both α- and β-ecdysone. While α-ecdysone was long considered the active moulting hormone of insects, 20-OH ecdysone proved to be more effective in most bio-assays (Ashburner & Richards, 1981).

The classical assumption has been that only source of the moulting hormone in insects is the prothoracic gland. However, recent information as reviewed by Gersch (1978) show that ecdysteroids are produced in the abdomens of ligated insects; moulting has occurred in insects of various species after extirpation of their prothoracic glands and that ecdysteroids have been found in the ovaries, eggs as well as embryos of insects. Further, the presence of ecdysteroids in oenocytes and pericardial cells has also been suggested.

In addition to zoo-ecdysteroids there are many phyto-ecdysteroids isolated from plant material especially fern and yerns. Metru and Horn (1980) gave a comprehensive list of zoo- and phytoecdysteroids with structures. Though it is now definitely established that the moulting hormones are biosynthesized in insects (Karlson & Hoffmeister, 1963; Nakanishi et al., 1972; Chino et al., 1974 and King et al., 1974), a second

question was raised as to why certain plants synthesize and accumulate such insect hormones. The answer to this question is that plants produce phytoecdysones for resistance to insect attack (Galbraith & Horne, 1966; William, 1970 and Harborne, 1977) but then insects must suffer from the disturbance of the hormonal balance by exogenous phytoecdysone. However, many plants which are now known as rich source of phytoecdysones are attacked by various insect pests, which survive without harmful effects. It is then possible that in insects some effecient defensive mechanism is operating against the exogenous phyto-ecdysones which may upset the hormonal balance. If such is the case the insects may either absorb no exogenous phytoecdysones or effeciently detoxicate them before/or after absorption.from the gut.

The moulting hormones and its related compounds which produce the effects of the prothoracic gland hormone as secreted by the living prothoracic gland, such as α-ecdysone, β-ecdysone, crusteodysone, ponasterone (A,B,C and D) etc. are steroids and non-specific.

The effect of application of exogenous moulting hormones on the growth and reproduction of insects has evoked consider-able interest. The primary effect of these hormones is on the moulting of insects. Moulting is a term that has caused con-siderable debate. Richards (1981) opined that the correct

usage implies two major events. The first, apolysis, is the detachment of epidermal cells from the old exoskeleton (Jenkin & Hinton, 1966). It is initiated by ecdysone (Madhavan & Schneiderman , 1968; Wigglesworth, 1973; Agui, 1977) and typically followed by production of an exuvial space, an ecdysial membrane, and a new cuticle (Locke, 1974 & 1976; Zacharuk, 1976). The second stage of the moult is ecdysis when the old exoskeleton is shed. The ecdysone concentration is almost zero immediately after the ecdysis, but increases after the middle of the instar reaching a maximum just before ecdysis.

Metamorphosis is a dramatic phase in the life cycle of holometabolous insects during which an extensive reconstruction of the basic body morphology takes place and the larva is transformed into the adult insect. As juvenile hormone is reported to have doubtful role in the metamorphosis of Diptera (Postlethwait, 1974), all these processes, directly or indirectly, are possibly induced and controlled by the hormone, 20-hydroxyecdysone (Kiss & Molnar, 1980).

The exogenous application of ecdysones and their analogues results in moulting disorders, suppression of metamorphosis, inhibition in ovarian development and oogenesis etc. in several insects. Among the modern approaches of insect control, ecdysones and their analogues enjoyed favourable consideration during the recent years and are grouped under what

are termed as 'third generation pesticides'.

In view of this exogenous moulting hormones (α-, β-ecdysone and 'Triol') were administered to Spodoptera litura, Diacrisia oblique and Dysdercus cingulatus to observe their effects on growth and reproduction of these insects in order to ascertain the possibility of hormonal control of these pests.

II. REVIEW OF LITERATURE

THYSANURA

Robbins et al. (1968) evaluated the effects of ingestion of α- and β-ecdysones and certain synthetic analogues on the growth and reproduction of Thermobia domestica. It was concluded, in general, that natural ecdysones were more active in moulting whereas their analogues were potent inhibitors of growth, metamorphosis and reproduction.

ORTHOPTERA

Although ecdysones from the prothoracic glands of insects are non-specific in action among the insects, their synthetic analogues may also show similar property. However, when a zooecdysone (obtained from crustacea = crusteodysone) from leaves of fern plant, Podocarpus gracilior, or plant growth regulator (Alar 85) were injected into newly ecdysed, gregarious fourth-instar larvae of Schistocerca gregaria, the morphometric ratios of the resulting adults were intermediate between the two extreme phases (El-Ibrashy et al., 1976). Further, in no case a complete transformation to the solitarious forms was obtained. Itthus showed that the above mentioned chemicals disturbed the normal metamorphosis in this species.

Joly et al. (1978) reported that implantation of pars intercerebralis and corpora cardiaca of supernumerary larvae of S. gregaria into immature females of this species induced precociously deposition of vitelline membrane. This also leads to the secretion of ecdysone in the ovaries at the end of oogenesis. But, injection of ecdysone into immature female resulted in hastening oocyte growth for 24 hours and then they degenerate. Similarly, in Carausius morosus an overload of β-ecdysone caused disturbances of the follicular cells activity involving malformations in the capitulum of the eggs (Mesnier & Thomas, 1981).

Injections of 20-hydroxyecdysone (1 ug/female) to newly emerged Acheta domestica imago stimulated oogenesis and fecundity, but higher doses (10 ug/female) inhibited them. Such an effect was observed in both intact and previously allatectomized crickets (Chudakova et al., 1982).

DICTYOPTERA

The effect of natural ecdysone in adult insects was first known in Leucophaea maderae when Engelmann (1959b) injected pupal ecdysone of Bombyx mori in adult L. maderae and found inhibition of oocyte growth.

Robbins et al. (1968 & 1970) evaluated the effect of ingestion of α- and β-ecdysones (natural ecdysones) and certain

synthetic analogues including 'Triol' on the growth and reproduction of Blatella germanica. They found that 37.5 ppm of 'Triol' completely inhibited the nymphal development. On the other hand, both α- and β-ecdysones were inactive even at 10 to 20 times the concentration of 'Triol' mentioned. Thompson et al. (1971) synthesized and evaluated the effect of two 5 β-hydroxy analogues of α-ecdysone. They found them to be the potent inhibitor of growth and reproduction of B. germanica. The effect of 20-hydroxyecdysone was studied upon the activity of corpora allata of the female Diploptera punctata. It was found that this ecdysteroid inhibited juvenile hormone biosynthesis by the corpus allatum, whether they were implanted into a male, or remained in situ within the female. Further, in the female this inhibition was reflected in reduced oocyte growth and vitellin content (Friedel et al., 1980).

HEMIPTERA

In Dysdercus cingulatus injection of β-ecdysone at 4.0 and 6.0 ug doses partially suppressed vitellogenesis and the number of eggs was reduced to about 50% of those of the control while the remaining eggs degenerated and resorbed (Jalaja et al., 1976).

Mansingh (1976) observed the effects of ecdysterone (β-ecdysone) on diapausing larvae and adults of Rhodnius prolixus. It was found that diapausing 5th instar larvae and adult females

suffered heavy mortality following the injection of 0.5-8.0 ug of ecdysterone. The treated insects were found to exhibit gradually increasing lethargy and metabolic exhaustion and became completely inactive 2 days before death. The ingestion of α-ecdysone by the female B. prolixus, however, inhibited oogenesis and such an effect was dose-dependent; doses higher than 4.0 ug ecdysone/mg body weight drastically reduced the size and shape of whole ovaries. On the other hand, Rankin and Jackle (1980) found that injection of ecdysone (α-ecdysone) or 20-hydroxyecdysone (β-ecdysone) into adult, male Oncopeltus fasciatus induced the appearance of both vitellogenin A and B in the haemolymph, suggesting the possible involvement of ecdysteroids in the control of vitellogenin synthesis in this species.

LEPIDOPTERA

Kobayashi and Burdette (1962) observed the effect of ecdysterone and inokosterone on the dauerpupae of Bombyx mori. They found that when 10 ug of ecdysterone or inokosterone was used, all pupae became pupal-adult mixtures without scale-hairs and scales. To see the effect of exogenous ecdysone on adult leg formation, 50 ug of ponasterone A was topically applied on the ventral surface of the thorax of B. mori pupae (Hagesawa & Ata, 1975). It was found that differentiation processes of pupal legs into adult ones were blocked resulting in malformed

legs. Earlier, Hagesawa and Ata (1971 & 1972) studied the penetrability of some phytoecdysones, ecdysterone, inokosterone, ponasterone A, and cyasterone through silkworm larval and pupal cuticle and their effect on pupal-adult development. It was found that the phytoecdysones penetrated the larval cuticle and penetration occurred effectively during the fourth instar, immediately after the fourth ecdysis and in mature larvae near the moulting and spinning period. When 25, 50 or 75 ug of ponasterone A and inokosterone were applied topically (Ventral side of the thorax) to each whole fresh female pupa, pupal-adult development was accelerated. The initiation of adult development signalled by apolysis and compound eye colouration occurred in the treated pupae 2 days earlier than control. The treated and control pupae emerged 12 to 15 and 15 days after pupation respectively. Comparisons between the effectiveness of ponasterone A and the sensitivity of the males and the females to both chemicals were done by topical application. Each chemical was applied to the same number of the male and the female pupae which received 25, 50 or 75 ug/pupa. The percentages of the emerging males and the females were almost the same in each treatment. However, the females had many more morphological defects (aberrant legs and antennae, defective eye colouration and deformed genitalia) than the males. Earlier works on the penetrability of the phytoecdysones through insect cuticle produced contradictory results. For examples, Sato(1968)

reported that phytoecdysones could penetrate through larval cuticle of some lepidopterous and other insects. But Ohtaki et al. (1967) and Robbins et al. (1968) found that phytoecdysones were ineffective when applied topically to certain insects e.g., flesh fly larvae and silkworm pupae.

Injection of exogenous β-ecdysone into isolated pupal abdomen of B. mori induced the initiation of ovarian development (Chatani & Ohnishi, 1976). The effectiveness of oral administration of phytoecdysones extracted from Chinese flora, was also tested in B. mori (Anonymous, 1979). It was found that feeding last instar larvae with mulberry leaves sprayed with cyasterone at a high dose (5 ug/larva) gave rise to intermediate larval-pupal forms, while treatment at a normal dose (1.5 ug/larva) resulted in the production of normal larvae. Kubo et al. (1981) investigated the dietary effect of 20-hydroxyecdysone and cyasterone on B. mori and Pectinophora gossypiella and found that insect death occurred at concentration of 50 ppm due to an anatomical inhibition of feeding caused by a moulting cycle failure. The moulting hormone activity of iso-inokosterone and inokosterone isolated from Achyranthis radix was examined using the pupae of B. mori (Kobayashi et al., 1967). Fifty-seven-day-old dauerpupae (artificially diapausing brainless paupae) were injected with 0.1 to 10 ug/pupa of iso-inokosterone or inokosterone. When 1.0 ug of either of these two hormones was injected into each of ten dauerpupae in two

groups, more than 90% of them emerged as normal moths in 14 to 20 days following the injection, whereas lesser amounts of these hormones did not show positive results (when 55% or more emerged) in this test. Injection of 10 ug/pupa of either hormone resulted in pupal-adult mixtures without scale-hairs and scales.

Martelli (1978) carried out large-scale laboratory experiments to observe the effects of ecdysteroids of plant origin (viz., α-ecdysone, β-ecdysone, makisterone A and muristerone) on post embryonic and adult stages of B. mori. These compounds were least active at application rates of 10-40 ug/cm^2 leaf of food plant. They also observed an anti-feeding effect with increasing doses of these compounds. Muristerone was the most effective in preventing completion of development. Further, it was observed (Anonymous, 1979) that adding the ecdysterone, extracted from plants, to the larval diet during the later stages of the fifth instar of B. mori resulted in early cocoon formation. The fourth and fifth instar larvae of the silk worm, B. mori were reared on artificial diet containing ponasterone A, ecdysterone and inokosterone. The growth of the larvae and their silk glands, fibroinsynthesizing activity and silk formation were investigated (Shigematsu et al., 1974). The fourth instar larvae were reared on the diet which separately contained either of 1.0,

2.5 and 5.0 ug of ponasterone A; or either of 5.0, 10.0 and 20.0 ug of ecdysterone; or each of 10.0, 20.0 or 40.0 ug of inokosterone/1 gm of dry powder of the diet. It was found that growth of the larvae decreased in the latter half of the instar and the period of the fourth instar was prolonged. The number of ecdysed larvae decreased with a diet containing 5.0 ug of ponasterone A. In contrast, the other two hormones did not cause retardation of growth, but 10.0 ug/g level of ecdysterone and inokosterone accelerated the growth. The diet for an assay of growth in the fifth instar, however, contained 2.5, 5.0, 7.5 or 10.0 ug/g of ponasterone A and ecdysterone, respectively. Inhibition of growth by ponasterone A doses was relatively greater with a rise of its concentration in the diets and no larvae matured and survived. On the other hand, diets which contained 5.0 or 7.5 ug of ecdysterone/g accelerated maturation of larvae, while the diet containing 10.0 ug of this hormone further increased the body weight of matured larvae. Further, the females which developed from fifth instar larvae were reared on a diet containing 5.0 ug of ponasterone A, 5.0 or 20.0 ug of ecdysterone, or 10.0 or 40.0 ug of inokosterone/g of dry weight. It was found that ponasterone A reduced the growth and caused the death of all larvae with a darkening of the skin. Only ecdysterone and ponasterone A caused a decrease in growth of the silk glands in the early half of the instar, and accelerated their growth during the last part of the fifth instar. Silk formation was much lower in larvae fed with diet

containing 5.0 ug of ecdysterone or 10.0 ug of inokosterone/ 1 gm of dry diet and was far greater in larvae fed with the diet containing 40.0 ug of inokosterone than in controls. To investigate the effects of exogenous and endogenous ecdysone prothoracic glands were eliminated in the decapitated larvae of B. mori by a second ligation between the prothorax and the mesothorax (Kimura, 1974). These second ligations were performed at 36, 48, 60 and 72 hours after decapitation. They were then injected with various doses (0.5-1.0 ug) of ecdysterone. Most of the re-ligated larvae, except the 72-hour-old larvae, could barely ecdyse to pupae unless they received ecdysterone. In this case the 36-hour-old larva required no less than 1.0 ug of the hormone to induce apolysis, but the 60-hour-old larva needed only 0.5 ug to obtain the similar effect.

Calvez (1981) confirmed the observations of Kijhout (1976) in Manduca sexta and that of Hwang Hsu et al. (1979) in Galleria sp. by showing that an ecdysone injection on the first day of the last larval instar of B. mori induced the synthesis of new larval cuticle. However, if the injection was made later, the new cuticle was pupal. Robbins et al. (1968) found that 'Triol', labelled as compound IV, at a dietary concentration of 37.5 ppm was almost inactive in M. sexta, though the same concentration severely inhibited development in houseflies. However, they noticed that the range of dietary concentrations of compound IV required to kill or inhibit development in 75% of

the test insects varied between 750 to 1000 ug/g. Hormonal control of epidermal detachment during the final feeding stage of M. sexta was observed by Riddiford and Curtis (1978). The abdominal epidermis was found to undergo a transient apolysis coincident with the first surge of ecdysone that initiated metamorphosis. During this detachment, the epidermal cells became committed to form pupal cuticle but morphological and histological studies indicated that larval endocuticle was still being deposited. Infusion of as little as 0.01 ug β-ecdysone over an 8-hour period into the larval abdomen isolated before the ecdysone surge was sufficient to initiate this detachment provided that juvenile hormone was absent. According to Nijhout (1976) slow infusions of β-ecdysone were more effective in eliciting a normal physiological response than mere discrete injections of the hormone. This was concluded after a series of experiments on M. sexta larvae. Abdomens isolated from large feeding larvae (weighing 8.0 to 9.5 gm) were infused with 30 or 40 ug/abdomen of β-ecdysone over 12 hours. Out of 28 abdomens treated in this manner 6 died and the remainder exposed their heart 12 to 18 hours after the infusion. A few individuals also showed a faint deposition of pink pigment along their dorsum. None of these abdomens deposited pupal cuticle even though many survived for more than 12 days after the infusion. Further, the infusion experiment was repeated on the abdomens of older larvae that had already exposed their heart. Each larva was infused with 30 ug of β-ecdysone over a period of 12 hours. Then out of 23 abdomens treated in this way,

3 died and the remainder transformed into perfect pupal abdomens approximately 3 days after the infusion. In an additional experiment, eight isolated abdomens of feeding larvae were induced to expose their heart by an infusion of β-ecdysone. Approximately 1 day after the heart became evident. Then they were infused once more with an additional dose of 30 ug of β-ecdysone over 12 hours. Five of these abdomens survived and deposited a perfect pupal cuticle 3 to 4 days after the infusion. However, when intact but paralysed final instar larvae, weighing 3 and 4 gm each, were infused with a 30 ug of β-ecdysone over 10 to 12 hours, a curious response was observed. Instead, a distinctive double row of dark brown spots appeared along the dorsum of all the 20 individuals subjected to this infusion. When small intact final instar larvae (weighing 1.5 to 2.5 g) were infused with 30 ug of β-ecdysone over 18 hours, they underwent analysis and deposited a perfect larval cuticle but no pupal structures were evident. Injection of a moderate dose of 20-hydroxyecdysone into pupae of M. sexta led to the prompt termination of diapause (Bradfield & Denlinger, 1980).

Graded doses of 6 phytoecdysones were compared with synthetic ∝-ecdysone in terms of their ability to provoke normal or abnormal development of diapausing pupae of the silkworm, Samia cynthia (Williams, 1968). The activity of the seven

hormones ranks in the following order: cyasterone> ponasterone B > ponasterone A> ponasterone C> α-ecdysone> β-ecdysone > inokosterone. All seven materials provoked normal adult development when injected in critical doses ranging from 0.2 ug (cyasterone) to 10 ug (inokosterone). These hormones, except α-ecdysone, caused extremely abnormal development when excessive doses were administered to pupae or isolated pupal abdomens. The typical result was the formation of naked or nearly naked non-viable moths which failed to complete adult development. It was, therefore, concluded that excessive ecdysone interfered with the cytological expression and implementation of genetic instructions for forming a normal moth. Kambysellis & William (1971) studied the role of ecdysone in the spermatogenesis of S. cynthia. Experiments were carried out on 20 insects 2 to 3 months after pupation. At zero hours each individual was injected with 20 ug α-ecdysone and after 1 hour the cysts obtained from the testes of the injected pupae showed no development. But after 24 hours the testes contained many cysts which had completed meiosis. By 48 to 72 hours after injection the testes contained steadily increasing number of cysts showing advanced spermatogenesis. Fujishita et al. (1982) studied the role of α-ecdysone and 20-hydroxyecdysone in the determination of gut-purge timing in Samia cynthia ricini. α-ecdysone at doses from 0.5 to 20 ug per larva of the last instar was injected during the period from 11:30 to 12:00 of

4th day following previous moulting. Injection of 10, 15 and 20 ug ecdysone caused precocious gut purge in more than 90% of larvae, which occurred during the period from 7 to 10 hours after injection (average 8.5 hours). Gut purge was thus accelerated by about 6.5 hours as compared to control larvae injected with distilled water.

Phytoecdysones (ponastesone A, inokosterone and ecdysterone) were extracted and applied as dips or topically to ligated larvae of Chilo suppressalis, Cadra cautella and Plutella xylostella (Sato et al., 1968). In Chilo all the steroids were tested by dipping experiments but on Cadra only ecdysterone was used in this manner. In these experiments it was recorded that ponasterone A was slightly more effective than inokosterone on last-instar larvae. Further, all the steroids at concentrations above 0.025% caused abdominal moulting in third and fourth instar larvae of Chilo. However, ecdysterone was effective on both species in the last instar. In the topical application tests, inokosterone and ecdysterone were applied to the abdominal terga of last instar of Plutella larvae and only ecdysterone to Chilo. Both the hormones were effective at the concentrations tested. Yagi et al. (1969) carried out the study to determine the effect of ecdysterone on the testes of diapausing larvae of C. suppressalis. From the remarkably rapid development of both testes and spermatocysts they concluded that ecdysterone promoted spermatogenesis of these larvae.

Imoto *et al.* (1982) studied the switchover from a larval to a pupal epidermal commitment on integument tissue fragments from early last-instar larvae (1-2 days after ecdysis) of *C. suppressalis* culture in Grace's medium containing 0.01 - 0.05 ug/ml 20-hydroxyecdysone for 24-72 hours. The degree of switchover increased with the duration of culture, as well as with the concentration of the hormone (upto 0.1 ug/ml). Above this concentration apolysis and new cuticle formation were induced without change in the epidermal commitment.

High doses of ∝-ecdysone and 20-hydroxyecdysone were shown to induce apolysis in diapausing larvae of *Ostrinia nubilalis* (Beck & Shane, 1969). Ingestion of ponasterone A blocked the development of cecropia larvae at a level of 1 ppb in a synthetic diet (Riddiford, 1970) whereas earlier report (Nakanishi, 1969) with lower doses of this hormone showed premature spinning. Waldbauer *et al.* (1978) reported that the injection of 10.0 ug/g pupae of *Hyalophora cecropia* usually caused hyperecdysonism and death before emergence.

Shibuya and Yagi (1972) observed the effect of ecdysterone on the cultivated ovaries taken from the last instar larvae of the greater wax moth, *Galleria mellonella*. They noticed that after 3 days, the ovaries enlarged in size and became transparent. Active contraction was observed in both ovaries and muscles attached to the ovaries. The effects of

α- and β-ecdysone or prothoracic glands on spermiogenesis was also seen on cabbage army worm, Mamestera brassicae and last instar larvae of the tobacco cutworm, Spodoptera litura in vivo. In testes cultures of these two species spermiogenesis was promptly accelerated with α-ecdysone or prothoracic glands, but no visible change occurred when β-ecdysone was added to the medium (Fukushima & Yagi, 1975).

Nowock (1972) studied the effect of α-ecdysone on the imaginal differentiation of the testes of Ephestia kuhniella as indicated by their fusion and torsion. Testes of pharate larvae of the penultimate instar and young pharate pupae were transplanted into isolated hindparts of 0 to 12 hours old pupae. Ecdysone was given by single or double (24 hours interval) injections of 0.5 ug/insect. It was found that both fusion and torsion could be induced in the transplants. Study was also made on the cocoon spinning behaviour of E. kuhniella and its correlation with 20-hydroxyecdysone injections (Ciebultowicz et al., 1980). In order to examine this, larvae were removed from their cocoon during the stage of outer envelope construction and injected with 20-hydroxyecdysone. It was found that they did not spin another outer envelope. Instead, they proceeded to construct the inner envelope. However, doses of the hormone which caused these effects were above the physiological level because most of the larvae moulted to larval-pupal intermediates. Some of them spun an abnormal cocoon or did not

spin at all, apparently as a result of the greatly accelerated pupal ecdysis. In the Mediterranean flour moth, Anagasta kuhniella, abdominal injection of 1 ug aqueous 20-hydroxy-ecdysone, any time prior to the initiation of sperm release from the testes, prevented the impending release of eupyrene sperm bundles (Thorson & Riemann, 1982). Further, Herman and Barker (1976) reported that in Danaus plexippus injection of low doses of β-ecdysone (.01 to 1 ug/female) inhibited ovarian development. However, higher doses (10 ug/insect) stimulated the male and female reproductive glands. According to them the ovarian inhibition resulted from decreased fat body vitellogenin synthesis. Further, the administration of β-ecdysone to the last instar larvae of Corcyra cephalonica induced premature pupation (Raghavan & Nadkarni, 1977). In the late larvae of the oleander hawkmoth, Deilephila nerii, injection of large doses (40-100 ug/larva) of β-ecdysone blocked or partially interfered with melanisation (Chang, 1978). From the diapausing slug moth pharate pupa, Monema flavescens, testes culture was maintained and when ecdysterone was added to the culture medium spermatocytes developed into spermatids (Takeda, 1972). It was further shown by Takeda (1978) that injection of β-ecdysone into the abdomen of diapausing prepupae of this species induced an extra moult and resulted in prepupal-pupal intermediates instead of normal pupae.

In Oncopeltus fasciatus, Rankin and Jackle (1980) studied the control of synthetic α- and 20-hydroxyecdysone on vitellogenin synthesis. They injected 0.5 ug of each ecdysone separately to a group of one day old females and thereafter at intervals of 2 days until day 10. A second group consisting of 10-12 day old males received injections of either 0.5 ug of α-ecdysone or 0.5 ug of 20-hydroxyecdysone and subsequently at intervals of 3-5 days up to 20 days. It showed that injection of α-ecdysone into females had no significant effect on the timing or the appearance of vitellogenin A in the haemolymph. However, in 15% of the males injected with α-ecdysone and 7% of those injected with 20-hydroxyecdysone a protein which appeared to be vitellogenin A was detected in the haemolymph 10 days after the first injection suggesting the possible involvement of ecdysteroids in the control of vitellogenin synthesis in this species.

The hormonal regulation of the larval diapause in the codling moth, Laspeyresia pomonella, was investigated by Sieber and Benz (1980). The larvae in diapause were injected with 0.5, 1.0 or 2.0 ug of 20-hydroxyecdysone. The responses following the injection were: (i) no reaction, the larvae remaining in diapause (ii) termination of diapause and pupation, and (iii) formation of an incomplete larval-pupal mosaic integument and apolysis, the insects not being able to shed the old

cuticle and dying a few days after this pathological moult. Browning (1961) reported that in diapausing pupae of Heliothis punctiger development was initiated following the injection of 20-hydroxyecdysone. The quantity of injected 20-hydroxyecdysone necessary to promote development in diapausing pupae varied from about 1 ug g^{-1} soon after pupation to 4 ug g^{-1} after 50 days.

DIPTERA

Ohtaki et al. (1967) assayed synthetic α-ecdysone and 6 ecdysones of plant origin and compared in terms of their ability to provoke puparium formation of isolated larval abdomens of Sarcophaga peregrina. All materials showed high activity in the following order : ponasterone A> cyasterone> β-ecdysone> ponasterone B> ponasterone C> α-ecdysone > inokosterone. Bodnaryk (1971) demonstrated the hormonal control of β-alanyl-L-tyrosin metabolism in the larva of the fly S. bullata by ecdysterone-injection experiments. Large amounts of ecdysterone caused precocious cuticle tanning in larvae and concomitant consumption of dipeptide.

Injection of 20 ug ecdysterone/larva in the 3rd instar of S. crassipalpis just after ecdysis or in the starved larvae caused precocious moult and formation of supernumerary larvae (Zdarek & Slama, 1973). The same treatment a few hours after

ecdysis lead to the formation of larval-pupal intermediates. Zdarek and Denlinger (1975) again observed the effect of two analogues, ecdysterone and 5 β-hydroxyecdysone, on the pupal diapause of S. grassipalpis. When small doses of ecdysterone (0.05 and 0.25 ug/pupa) were injected twice or three times into the same pupa at 3-day intervals, adult development resumed earlier than in pupae that received the same net amount of hormone in one dose. High doses (1.0 ug/pupa) of these hormones caused immediate termination of diapause but adult morphogenesis is prolonged in direct proportion to the dose of the ecdysoid. Flies receiving high doses of ecdysoids were characterized by developmental abnormalities such as underdeveloped antennae, compound eyes and mouth parts. However, in S. argyrostoma pupae, injection of 6-10 ug/g β-ecdysone led to hyperhormonal abnormalities affecting antennae, genitalia and bristle orientation (Gibbs, 1976). Fraenkel and Hollowell (1979) reported that injection of 20-hydroxyecdysone (5 ug/fly) to adult Phormia regina or S. bullata, when primary oocytes started to develop, caused the primary oocytes to degenerate, with concomitant development of the secondary oocytes.

On Musca domestica larvae the effect of certain natural ecdysones as well as synthetic analogues given through diet was studied on the development and growth (Robbins et al., 1968). Compound IV, one of the analogues, severely inhibited development at concentrations as low as 37.5 ppm. Other analogues

labelled as V and VI and ponasterone A (phytoecdysone) were one-fourth as active as compound IV whereas 20-hydroxyecdysone was inactive even at the highest concentration tested (i.e. 150 ug/g). Compound IV, 20-hydroxyecdysone and ponasterone A, all inhibited ovarian maturation and egg production when fed in an artificial diet to newly emerged houseflies. Concentrations of 0.1% of compound IV severely inhibited ovarian growth showing shortening of terminal oocyte as compared to normal in about 80% of the flies while concentrations as low as 0.01% interfered with normal development of oocytes. Bowers (1968) recorded that 'Triol' and a synthetic juvenile hormone applied with a synergist was more effective to inhibit the development. Further, Thompson et al. (1971) evaluated the effect of two 6 β-hydroxy analogues of ∝-ecdysone, labelled as VIIIa and VIIIb, on the growth and reproduction of M. domestica. They found that ingestion of compound VIIIa at 150 ppm concentration inhibited the larval development of houseflies by 95%, whereas this inhibition was only 32% by VIIIb. The percent inhibition of ovarian development at 0.5% concentration of compound VIIIa was 17% but compound VIIIb had no effect on ovarian development. Further reproduction i.e., production of viable eggs was inhibited by 32% by compound VIIIa at 0.10% concentration. However, compound VIIIb was ineffective at this concentration. According to Kaplanis et al. (1971) if ecdysone analogues like

22,25-bisdeoxyecdysone (BDE) were applied topically in combination with certain synergists such as sesamax and piperonyl butoxide had enhanced inhibition on the ovarian growth of house flies. Contrary to the observation of Robbins et al. (1968) on the housefly, Singh and Russell (1980) observed that the ingestion of 20-hydroxyecdysone by the housefly larvae caused adverse effect on growth and development. They recorded that at a concentration of 100 ppm in a Casein diet, this hormone was found to be toxic causing 84% larval mortality whereas the same concentration in an amino acid diet caused 65% larval mortality but the rate of larval development was greater than the control. Further, some puparia showing prothetely were produced at all concentrations tested. In the subsequent experiments, Singh et al. (1982) evaluated and compared the effect of 20-hydroxyecdysone (β-ecdysone) and 5,20-dihydroxy-ecdysone (analogue) incorporated into the larval diet of M. domestica. They found that the former ecdysone caused 84% and the latter 77% larval mortality at 100 ppm concentration. The effect of these ecdysones which they extracted from plants were greater than ponasterone C, makisterone A and dacrysterone.

The effect of two phytoecdysones --ecdysterone and cyasterone was studied on the imaginal discs of Drosophila melanogaster (Postlethwait and Schneidermann, 1968 & 1970). Imaginal leg discs from mature larvae were transplanted into

the abdomens of fertilized adult female flies. It was found that the injection of large doses of ecdysterone (over 1200 ug/g) into the hosts caused complete metamorphosis of transplanted imaginal discs. They also showed that cyasterone was a more potent moulting hormone than ecdysterone. Gvozdev et al. (1974) reported that β-ecdysone and 2-deoxy-α-ecdysone (analogue) specifically inhibited the growth of the established cell lines of D. melanogaster. Further, according to Berger et al. (1978) permanent cell lines derived from embryonic stage showed pronounced morphological and biochemical changes in response to the addition of β-ecdysone or its various analogues used. Maslennikova and Luchnikova (1979) added various amounts of ecdysone to the food of adult D. melanogaster, resulting from the larvae reared on a standard medium. When this hormone was added to a special cholesterol free diet at a daily rate of 1-10 ug/adult, it increased average daily female fecundity to the level of that observed in those flies which were fed on a normal diet. Such stimulation in the fecundity of the flies was only observed at the beginning of the reproductive cycle up to 3-4 days after eclosion. Chudakova et al. (1982) studied the effect of 20-hydroxyecdysone on the fecundity of D. melanogaster. It was found that the addition of 20-hydroxyecdysone to steroid defecient diet in dose 10.0 ug/female increased the female fecundity up to 16% as compared to that of control.

Wright and Kaplanis (1970) observed the effect of feeding of 3 natural ecdysones (α-, β-, and ponasterone A) and 3 synthetic analogues (labelled as IV, V and VI) on the fecundity of stable fly, Stomoxys calcitrans. When 1 day old female stable flies were put on citrated bovine blood with 0.1% -ecdysone for 5 days, egg production was definitely inhibited but the inhibition was not complete. Further, such flies oviposited at 13th day after emergence instead of at 6th day in the normal flies. The presence of 20-hydroxyecdysone in the diet of the newly emerged stable flies also inhibited egg production completely. Further, it also severely inhibited growth of ovaries. Ponasterone A and ecdysone analogues caused little inhibition. The effect of ingestion of 20-hydroxy-ecdysone on the ovarian maturation of S. calcitrans was re-evaluated by Wright et al. (1971). According to their observation young female stable flies were permanently sterile after having ingested a 0.1% solution of 20-hydroxyecdysone in fresh citrated beef blood for four consecutive days. It was also found that this ecdysone prevented the synthesis of lipid materials necessary for vitellogenesis and final egg maturation.

Thompson and Horn (1969) injected crustecdysone, α-ecdysone and callinecdysone A in the third instar larvae of blowfly, Calliphora styria to observe their effect on puparium formation of this genus. Administration of either of these

hormones at a dose of 0.2 ug/larva produced a marked acceleration of puparium formation. Higher doses of these steroids (1-2.0 ug/larva) resulted in sclerotization of the majority of the larvae without normal contraction to the ovoid puparial form. The cuticle, although of the usual red-brown colouration, remained relatively thin, soft and crinkled in these insects. Callinecdysone A (from Calliphora) and crustecdysone (from Crustacea) appeared to act additively in producing this precocious sclerotization. In some high dose group, a few larvae formed normal puparia. Galbraith et al. (1975) synthesized and assayed the biological activity of 2,25-dideoxy-α-ecdysone and deoxyecdysone (both analogues) on Calliphora. They found that the latter ecdysone was as active as that of the natural material and equal to that of β-ecdysone on the ovarian maturation whereas the former ecdysone was only half active as β-ecdysone and only slightly more so than the natural material.

Robbins et al. (1970) observed the dietary effect of ecdysones and ecdysone analogues on the larval development of yellow fever mosquito, Aedes aegypti. Ponasterone A at 1.0 ppm concentration caused 60% inhibition in larval development whereas all other analogues (I, IV, V, IX and X) tested caused 100% inhibition at the same concentration (1.0 ppm). Further, the effect of two 5 β-hydroxy analogues of α-ecdysone, labelled as (VIIIa) and (VIIIb), on the growth and reproduction was

observed by feeding them to the larvae of A. aegypti (Thompson et al., 1971). They found that the ingestion of either of these two compound at 1.0 ppm concentration caused 100% inhibition of larval development. Further, Fallon et al. (1974) recorded that when β-ecdysone was injected to the adult female, A. aegypti without a blood meal, it induced the synthesis of yolk protein (vitellogenin). After injection of 5 ug ecdysone per mosquito, vitellogenin constituted 80% of the total protein secreted by explanted fat body, a proportion comparable to that produced by fat-body from blood-fed females. Response to ecdysone is dose-dependent : 0.5 ug/female was required to stimulate synthesis to 50% of the level found 18 hour after a blood meal.

The oocytes of 3-day old unfed A. aegypti mosquitoes, usually in a state of oogenic arrest, was stimulated and variable amounts of yolk were accumulated following the injection of microgram doses of ecdysterone (Raikhel and Lea, 1982). They also found that these doses also induced the deposition of plaques of vitelline envelope by the follicle cells.

When female Culex tarsalis were treated topically with β-ecdysone or 22-isoecdysone before a blood meal, eggs laid were viable. If similar treatments were carried out after a blood meal, however, eggs laid by the females treated with β-ecdysone were effectively infertile (Ittecheriah et al.,1974).

On the other hand, injections of ecdysterone at a dose of 5-10 ug per female into unfed females of Culex pipiens pallens, 1-3 day after emergence, initiated increased growth of the ovarian follicle and yolk deposition (Meixun et al., 1980). In Anopheles stephensi, vitellogenic primary-follicle development from the resting stage was not induced by the injection of 20-hydroxyecdysone (Redfern, 1982). However, there was premature growth of the penultimate follicle and induction of yolk-protein synthesis.

HYMENOPTERA

In hymenopterous insects effect of ecdysone was only studied on a parasite wasp, Nasonia vitripennis by Loof et al. (1979). According to them in the diapausing wasp, the diapause was easily terminated by topical application of ecdysterone (β-ecdysone). This suggested that this hormone had growth promoting factor.

COLEOPTERA

The effect of natural and synthetic ecdysteroids on coleopterous species has been studied in only a few species and most of the information has been contributed by Robbins and his colleagues.

In Tribolium confusum (flour beetle) development and reproduction was studied following the feeding of certain natural and synthetic ecdysteroids to the newly emerged aadult beetles (Robbins et al., 1968). It was found that by feeding 1.0 percent of compound IV (an analogue) to the mixed group of sexes, there was 97% reduction in the progeny. But when the males and the females were fed separately on this compound IV, only the female reproductive system was affected, which showed shortening of the vitellarium and oocytes. However, 20 hydroxy-ecdysone was less than one-tenth as active as compound IV in inhibiting the reproduction. It was further established that the doses of compound IV ranging from 500 to 750 ug/g body-weight were sufficient to kill or inhibit development in 75 percent of the insects. Later, Robbins et al. (1970) fed ecdysones and synthetic analogues to the larvae of T. confusum to observe their moulting activity and inhibitory effects on their growth, metamorphosis and reproduction. Each of α-ecdysone, ponasterone A and cyasterone in 0.50% concentration in the larval diet caused 28%, 100% and 16% inhibition of development respectively. Similar concentration of the analogues labelled as compound I, IV, V, X respectively caused 100%, 100%, 70% and 69% inhibition in development. Further, effect of these ecdysones and synthetic analogues on the reproduction was observed by feeding each of them in the adult diet at 1.0% concentration for 10 days. Out of the several

ecdysones tested only 20-hydroxyecdysone appreciably inhibited reproduction whereas ponasterone A caused about 45% mortality. Among the synthetic analogues compound IV also caused mortality and severely inhibited reproduction in the surviving insects. Aside this, synthetic compounds I, VII and X produced greater than 90% reproductive inhibition while compound I caused 70% reduction of progeny at 0.5% concentration but it was inactive at 0.1%.

Adult female Anthonomous grandis became permanently infecund when they were fed on various analogues of ecdysone (Earle et al., 1970). A trihydroxy 6-keto steroid ('Triol') was found to be particularly effective and was evaluated as female chemosterilant and 0.1% 'Triol' almost completely inhibited the egg production. They also found that feeding of 3 ppm of 'Triol' lowered the yield of adults but 10 ppm almost eliminated the formation of adults.

Walker and Thompson (1973) studied the pathological effects of 22,25-bisdeoxyecdysone on Mexican bean beetle, Epilachna varivestis. Newly ecdysed larvae of each stage were confined with the leaves treated with 100, 500, or 1000 ppm of this hormone. Growth and feeding of larvae of stages 1-3 did not appear to be inhibited when they were confined with 1000 ppm concentration. However, they were unable to moult, or died shortly after ecdysis. One 2nd instar formed a super-

numerary instar with extended wing pads, but was unable to form a normal pupa at the next ecdysis. Fourth instars were more sensitive to this ecdysone than the other instars. Those treated with 1000 ppm abbreviated the pupal period and all treated larvae had attempted ecdysis within 4-5 days compared with the 7-day period for the control. Many of the resulting neotenic forms partially or totally shed the exuviae and some of them had short, fully extended wing pads. Nearly mature 4th instars were unaffected by foliar application of 100 ppm and topical applications of as much as 10 ug applied to mature prepupae or to 0- to 1-day old pupae had no effect on emergence of normal appearing adults.

According to Socha and Sehnal (1972) injection of 20 ug ecdysone to each pupa of Tenebrio molitor hastened the adult emergence which took place within 90 to 120 hours instead of 144 to 168 hours. However, doses of ecdysterone exceeding 0.02 ug caused formation of pupal-adult intermediates. On the other hand, Laverdure (1975) observed that in T. molitor, the presence of the prothoracic glands inhibited ovarian development in pupa. Although in vitro cultures of ovaries had shown that 3 ug of β-ecdysone/ml stimulated the growth of oocyte in very young ovaries but the development was abnormal. Experiment with β-ecdysone showed that this hormone induced normal develop-

ment in pupal ovaries, stimulating cell division and ensuring oocyte development as well as follicle organisation. It is 10 times more active than α-ecdysone in stimulating ovarian development and 30 times more active in maintaining it at later stages.

III. MATERIALS AND METHODS

1. Breeding and maintenance of stock culture:

(a) Spodoptera litura Fabr.

For the stock culture of this species, adult moths were collected in the night around the lamp posts in the Aligarh Muslim University Campus during the months of July and August. They were kept in all glass circular rearing jars measuring 20 x 15 cm with their bottoms filled with sterilized and damp sand about 5 cm in thickness. The top of these jars was covered with muslin. These jars were maintained at 30 ± 1°C and 70-80% relative humidity in controlled temperature cabinet.

The moths were fed on saturated glucose solution (Hashmat & Khan, 1970). For this purpose a piece of cotton wool soaked with glucose solution was wrapped around a glass slide which was obliquely inserted in the damp sand. Apart from this, few strips of white paper were placed in the jar for the females to oviposit on them. Oviposition took place within 3-5 days in batches. After oviposition adults were transferred to fresh jars. The eggs hatched within 4-6 days. The larvae were reared on fresh and young castor leaves. From this culture 5th and 6th instar larvae of the desired age were sorted out for the experiment.

(b) Diacrisia obliqua Wlk.

Adult moths of this species were collected around the lamp posts in the month of December. These adults were maintained in glass rearing jars measuring 20 x 15 cm in size. The bottom of these jars were filled with sterilized and damp sand about 5 cm in thickness. The open top of each jar was covered with a piece of muslin which was fixed by means of a rubber band. These rearing jars were maintained at a temperature of 29 ± 1°C and 70-80% relative humidity.

Adults moths were fed on 5% glucose solution. Egg laying took place within 4-6 days, after which the adults were transferred to separate jars. The eggs hatched within 5-10 days. The larvae were then reared on fresh and young castor leaves. From this stock 5th and 6th instar larvae of required age were sorted out for experiment.

(c) Dysdercus cingulatus Fabr.

The adults and various nymphal stages of this species were collected from the cotton crops during the Kharif period and maintained in glass rearing jars measuring 20 x 15 cm with 5 cm thick, damp and loose sand at the bottom. The jars were kept at 29 ± 1°C and 70-80% relative humidity. These insects were daily fed on soaked healthy cotton seeds. The females laid eggs within 3-4 days in clusters on the moist sand. After oviposition the adults were transferred to fresh jars.

These eggs hatched within 5-7 days. Newly emerged nymphs were provided with soaked cotton seeds as food. From this nymphal stock, 4th and 5th instar nymphs were sorted out for experiment.

2. Experimental procedure:

(i) Preparation of stock solution

In the present investigation effect of α- and β-ecdysones (Simes, s.p.a., Milan, Italy) and 'Triol' (Courtsey: M. J. Thompson, USDA, U.S.A.) was studied by applying their different doses on the above mentioned insects. The different doses applied were 0.5, 1.0, 2.0, 4.0 and 6.0 ug per larva/nymph of either instar. For that 15 mg of either of these ecdysones was dissolved in 2.5 ml acetone: water (3:1) to obtain the stock solution, S. Now 1 ul of this solution contained 6.0 ug ecdysone. Thus doses of 6.0 ug ecdysone per larva/nymph were given by applying 1 ul of this stock solution. Then 1 ml of this solution (s) was taken and diluted by adding 0.5 ml of acetone so that solution S_1 was obtained. 1 ul of this solution (S_1) contained 4.0 ug ecdysone. Again 0.5 ml of solution S_1 was further diluted by adding 0.5 ml of acetone to obtain 1 ml of solution S_2. 1.0 ul of this solution contained 2.0 ug ecdysone. For 1.0 ug dose 0.5 ml of solution S_2 was further diluted by adding 0.5 ml of aceton to obtain 1 ml of solution S_3. Further, when 0.5 ml of solution S_3 was

diluted by adding 0.5 ml of acetone to obtain 1 ml of solution S_4. 1.0 ul of this solution contained 0.5 ug ecdysone.

(Note: In the present text wherever ug or ul has been typed it respectively stands for µg and µl).

(ii) Methods of application

The 5th and 6th instar larvae (24 hours old) of Spodoptera litura and Diacrisia obliqua were injected/fed with β-ecdysone or 'Triol' and α-ecdysone or 'Triol' respectively. In Dysdercus cingulatus, 4th and 5th instar nymphs (24 hours old) were treated with α-ecdysone and 'Triol' by injection or topical method.

Injection of the hormone in S. litura and D. obliqua was made by means of a tuberculine syringe through lateral intersegmental membrane between metathorax and first abdominal segment of the larvae. But in D. cingulatus, hormone was injected into the last abdominal segment.

To observe the effect of ingestion of the respective hormones by the larvae of S. litura and D. obliqua castor leaves (5 x 5 cm) sprayed with these hormones were fed to the 5th and 6th instar larvae of these insects. For that the larvae were previously starved overnight.

Topical application was made only in D. cingulatus. The ecdysone was applied on the thorax of newly moulted 4th

and 5th instar nymphs.

Each dose was tested against 100 larvae/nymphs of these insects by either method and replicated 5 times. The larvae/nymphs receiving 1.0 ul acetone only served as parallel control.

3. Methods for observations on growth and reproduction:

After the treatment by injection, feeding or topical method, the larvae/nymphs were kept at the above mentioned temperature and humidity. They were then observed for larval/nymphal mortality, longevity, formation of supernumerary larvae, larval-pupal intermediates, pupal mortality and malformation, adult emergence and abnormal adults.

To observe the effects of these hormones on the fecundity and fertility of the females emerged from the treated larvae/nymphs, 6 females were paired separately with equal number of males of corresponding age obtained from the normal stock. Number of eggs laid by each female was recorded. These eggs were then transferred to the damp sand surface of fresh jar for hatching. A parallel control consisting of the females and males both emerging from the untreated larvae was also maintained.

After hatching, the larvae from the eggs of the females affected by each treatment were collected in 70% alcohol for counting. The unhatched eggs were also collected as dried and

counted separately.

4. Statistical analysis:

The data were analysed statistically. Standard deviation was calculated by the following expression:

$$S.D. = \sqrt{\frac{\leq D^2}{n-1}}$$

where,

S.D. = Standard deviation

$\leq D^2$ = Sum of square of the differences of mean value

n = Number of observations

On the basis of S.D., standard error was also calculated by the following formula:

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

where,

S.E. = Standard error

S.D. = Standard deviation

n = Number of observations

For significance test following formula was applied:

$$t = \sqrt{\frac{m_1 - m_2}{\frac{SD_1}{n_1} + \frac{SD_2}{n_2}}}$$

where,

t = Significant value

$\bar{x}_1$ = Mean of first set of observation

$\bar{x}_2$ = Mean of second set of observation

$S.D._1$ = Standard deviation of first set of observations

$S.D._2$ = Standard deviation of second set of observations

n_1 = Number of observations of the first set

n_2 = Number of observations of the second set.

The calculated 't' was compared with the tabulated 't' (Bailey, 1959) at 5% level. If the former value is higher than the latter, the data is significant otherwise it is insignificant. The tabulated 't' value at 5% level is 2.57.

Note: In the present text wherever ug or ul has been typed it respectively stands for µg and µl.

IV. RESULTS AND OBSERVATIONS

1. Spodoptera litura:

(A) Effect of injection of different doses of β-ecdysone on 5th instar larvae of Spodoptera litura:

Doses : 0.5, 1.0, 2.0, 4.0 and 6.0 ug/larva

Fifth instar larvae, 24 hour old, were sorted out from the stock culture and injected with different doses of β-ecdysone. The larvae injected with 1.0 ul acetone per larva served as parallel control.

In the first test set, 100 larvae were injected with 0.5 ug β-ecdysone per larva (Table - 1). It was found that 2 larvae died within 24 hours after the treatment and 3 of them died at next larval-larval moult. However, the morphology and longevity of the 5th instar were not affected. The remaining larvae normally moulted to 6th instar. Further, 3 of the 6th instar larvae died soon after moulting from 5th instar whereas 2 larvae died later at larval-pupal moult. The morphology and longevity of the 6th instar larvae were also unaffected. The remaining larvae pupated. All the pupae were normal and successfully metamorphosed to adults. The adults were perfectly normal. The number of adults was, however, 5% less as compared to control. For observations on fecundity and fertility 6 females emerged from the treated 5th instar larvae (affected

females) were paired separately with normal males (emerging from untreated larvae) of corresponding age. Each of the affected females, on average, laid 136.16 eggs less than that of control. The percentage of egg hatching was also reduced by 1.99 percent as compared to control (Table - 2). The fall in fecundity ($t = 2.51$, $p > 0.05$) was insignificant but in fertility ($t = 2.76$, $p < 0.05$) it was statistically significant.

Further, in the second group of 100 test larvae, 1.0 ug β-ecdysone was injected in each larva (Table - 1). Among them 3 larvae died 20 hours after treatment and 3 of them died at next larval-larval moulting. The morphology and longevity of the larvae were not affected. When these larvae moulted to 6th instar, 4 larvae died at various intervals of time and 2 of them died at larval-pupal moult. The 6th instar larvae also maintained normal morphology and longevity. Pupation was normal and all the pupae successfully transformed into adults. The adult emergence was 7 percent less than that of control. All the adults were normal. Further, 6 affected females were paired separately with normal males of corresponding age for observation on fecundity and fertility. Each of the affected females, on average, laid 384.16 eggs less than that of control. The egg hatching was also reduced by 10.6 percent as compared to control (Table - 2). The observation on fecundity ($t = 1.43$, $p > 0.05$) was insignificant but fertility ($t = 4.92$, $p < 0.05$) was

found to be statistically significant.

Further, in the next group of 100 test larvae, each was injected with 2.0 ug β-ecdysone (Table - 1). Out of these treated larvae, 5 larvae died during the larval duration of the same stage whereas 5 larvae suffered mortality at larval-larval moult. However, the morphology and longevity of these larvae remained unaffected. The remaining larvae moulted to 6th stage. Among the moulted larvae, 2 died soon after moulting and 2 of them died later. The remaining larvae then proceeded for pupation but 4 of them died at larval-pupal moult. The pupae were normal and all of them successfully metamorphosed to normal adults. In comparison to control the adult emergence was, however, 13 percent less. Further, 6 affected females were paired separately with normal males of corresponding age for observing fecundity and fertility. Each of the affected females, on average, laid 789.83 eggs less than that of control. The egg hatching was also reduced by 29.37 percent as compared to control (Table - 2). The observation on fecundity ($t = 7.09$, $p<0.05$) was highly significant but fertility ($t = 1.47$, $p>0.05$) was not statistically significant.

In the fourth group of 100 larvae, 4.0 ug β-ecdysone was injected in each larva. Among the treated larvae, 5 larvae died soon after injection whereas 3 larvae died later. The larvae did not show any morphological defect. They also main-

tained normal larval duration. The larvae then moulted to 6th stage but 8 of them died in the process of larval-larval moulting. The morphology and longevity of the 6th instar were also normal. Further, 10 larvae died during the 6th stage whereas 9 larvae failed to cast off their exuviae at larval-pupal moulting and died. The remaining larvae normally pupated. The pupae were normal and all of them successfully metamorphosed to adults. The adults had no abnormal features. Further, 6 affected females were paired separately with normal males of corresponding age for observing fecundity and fertility. Each of the affected females, on average, laid 1492.6 eggs less than that of control. The egg hatching was 20.95 percent less as compared to control (Table - 2). The reduction in fecundity ($t = 1.68$, $p > 0.05$) and fertility ($t = 2.02$, $p > 0.05$) was statistically insignificant.

In the fifth group of 100 test larvae, each larva was injected with the strongest dose (6.0 ug) of β-ecdysone. Then, 10 larvae died half an hour after injection and 2 larvae died later. There was no change in the morphology and longevity of the surviving larvae. Further, 8 larvae suffered mortality at moulting from 5th to 6th instar and 10 larvae died soon after moulting to 6th instar. Among the remaining 6th instar larvae, 4 died later. The morphology and duration of these larvae were normal. Again, 10 larvae suffered mortality at larval-pupal

moulting. The remaining 56 larvae pupated normally. All the pupae except 3 metamorphosed to normal adults and adult emergence was 42 percent less than that of control (Table - 1). Then, 6 affected females were paired separately with normal males of corresponding age for observing fecundity and fertility. Each of the affected females, on average, laid 1638.5 eggs less than that of control. The egg hatching was reduced by 60.6 percent as compared to control (Table - 2). These observations on fecundity ($t = 3.89$, $p<0.05$) and fertility ($t = 8.26$, $p<0.05$) were statistically highly significant.

(B) Effect of injection of different doses of β-ecdysone on 6th instar larvae of Spodoptera litura:

From the stock culture 6th instar larvae, 24 hour old, were sorted out for injection of different doses of β-ecdysone (0.5, 1.0, 2.0, 4.0 and 6.0 ug per larva). The larvae injected with 1.0 ul acetone only served as parallel control. Each dose was tested against 100 larvae.

In the first experiment 0.5 ug β-ecdysone was injected to each larva. Then 6 larvae died within 20 hours after injection. The survived larvae showed no change on their morphology and longevity. Later, 6 larvae died in the process of larval-pupal moulting. The pupae were normal and all of them transformed into adults. As compared to control the adult emergence was reduced by 2 percent (Table - 3). Further, 6

females emerged from the treated larvae were paired with males of untreated stock (normal) of corresponding age to observe fecundity and fertility. Each of the affected females, on average, laid 146.0 eggs less than that of control. The egg hatching was also reduced by 3.61 percent as compared to control (Table - 4). The observation on fecundity ($t = 2.29$, $p > 0.05$) and fertility ($t = 2.47$, $p > 0.05$) was statistically insignificant.

In the second group of 100 larvae, each larva was injected with 1.0 ug β-ecdysone. Then 2 larvae showed lethargic conditions 2 hours after injection and ultimately died whereas 6 more larvae died later. There was, however, no change in the morphology and longevity of the survived larvae. Among the remaining larvae 6 died at the larval-pupal moult. Pupation was normal and all except 4 normal pupae successfully transformed into normal adults. The adult emergence was 8 percent less as compared to control (Table - 3). Further, 6 affected females were mated separately with normal males of corresponding age to observe fecundity and fertility. Each of the affected females, on average, laid 490.67 eggs less than that of control. The egg hatching was reduced by 10.3 percent as compared to control (Table - 4). These observations on fecundity ($t = 4.58$, $p < 0.05$) and fertility ($t = 4.48$, $p < 0.05$) were significant.

In the third experiment, 100 larvae were taken and each larva was injected with 2.0 ug β-ecdysone. Then, 5 larvae showed lethargic conditions half an hour after injection and ultimately died whereas 8 other larvae died later. This dose also did not produce any adverse effect either on morphology or longevity of the larvae. Further, 8 larvae died in the process of larval-pupal moulting. Pupae were normal and all except 5 normal pupae transformed into adults. Thus, the adult emergence was 16 percent less as compared to control (Table - 3). Then 6 affected females were paired with normal males of corresponding age for observing fecundity anf fertility. Each of the affected females, on average, oviposited 890.5 eggs less than that of control. The egg hatching was also reduced by 8.44 percent as compared to control (Table - 4). These observations on fecundity ($t = 5.98$, $p<0.05$) and fertility ($t = 6.63$, $p<0.05$) were statistically significant.

In the fourth group of 100 larvae, each larva was injected with 4.0 ug β-ecdysone. Out of these 9 larvae developed acute lethargic conditions soon after injection and ultimately died whereas 4 other larvae died later. The morphology and longevity of these larvae remained unaffected. Further, 8 larvae attempted to moult to extra-instar but died in the process. Thereafter 10 larvae died at larval-pupal moulting. The remaining larvae pupated. However, all the pupae were not

normal. Among the pupae 7 normal and 2 abnormal pupae could not metamorphose to adults. The abnormal pupae had larval head and abdominal legs. The adult emergence was 30 percent less than that of control (Table - 3). However, all the adults were perfectly normal. Further, 6 females emerged from the treated larvae were paired separately with normal males of corresponding age to observe fecundity and fertility. Each of the affected females, on average, laid 1444.0 eggs less than that of control. The egg hatching was also reduced by 47.64 percent as compared to control (Table - 4). These observations on fecundity ($t = 3.95$, $p < 0.05$) and fertility ($t = 5.83$, $p < 0.05$) were statistically significant.

In the fifth group of 100 test larvae, each larva was injected with the strongest dose (6.0 ug) of β-ecdysone. Again 12 larvae developed acute lethargic conditions and ultimately died whereas 5 more larvae died later. The injection caused no effect on the morphology of the larvae. However, the longevity of the larvae was shortened by 20-24 hours. It was also found that 12 larvae attempted to moult to extra instars but 10 of them died in the process whereas 2 larvae successfully transformed into supernumerary larvae (Fig. 1A). The extra instars thus formed showed acute lethargic conditions and subsequently died. The average length and breadth of these larvae were 4.80 cm and 0.70 cm respectively as compared to 4.30 cm and

0.60 cm of normal 6th instar larvae. These larvae, on average, weighed 0.82 gm whereas normal 6th instar larvae weighed 0.635 gm (average). The remaining larvae, except 13 which died at larval-pupal moult, pupated. Among the pupae which failed to metamorphose to adults were 10 normal and 3 abnormal pupae. The abnormal pupae had larval head and abdominal legs. The emergence of adults was 45 percent less than that of control (Table - 3). When 6 affected females were paired separately with normal males of corresponding age for observing fecundity and fertility, each of the affected females, on average, laid 1653.0 eggs less than that of control. The egg hatching was also reduced by 70.15 percent as compared to control. These observations on fecundity ($t = 5.02$, $p<0.05$) and fertility ($t = 7.97$, $p<0.05$) were statistically highly significant.

(c) <u>Effect of ingestion of different doses of β-ecdysone on 5th instar larvae of Spodoptera litura</u>:

Individual larva of 5th instar, 24 hours old and starved overnight, was separately allowed to feed on a known size of castor leaves (5 x 5 cm) sprayed with one of the different doses (0.5, 1.0, 2.0, 4.0 and 6.0 ug per larvae) of β-ecdysone. Thus each dose was fed to 100 larvae. Similarly, 100 larvae were fed on leaf pieces treated with 1.0 ul acetone only for parallel control.

In the first group of 100 larvae, each larva ingested 0.5 ug β-ecdysone. Then 5 larvae died 12 hours after ingestion. However, ingestion of this dose did not affect either morphology or longevity of the larvae. The larvae normally moulted to 6th instar stage and they did not suffer mortality at larval-larval moult. The morphology and longevity of the larvae moulted to 6th instar also remained unaffected. However, 2 larvae died during 6th instar whereas 1 larva died at larval-pupal moult. The remaining larvae pupated normally and all of them successfully metamorphosed to adults. The adult emergence was 8 percent less than that of control (Table - 5) but the adults were normal. Further, 6 affected females were separately paired with normal males of corresponding age. Each female, on average, laid 477.5 eggs less as compared to control. The percentage of egg-hatching was, however, 1.29 more than that of control (Table - 6). These observations on fecundity ($t = 4.45$, $p<0.05$) and fertility ($t = 3.83$, $p<0.05$) were statistically significant.

In the second group of 100 larvae, each larva was fed 1.0 ug β-ecdysone. Then 2 larvae died soon after ingestion of this dose and 3 larvae died later. There was no change in the longevity and morphology of the surviving larvae. Again 1 more larva died in the process of moulting to 6th stage. Further, 3 larvae of the 6th stage died at various intervals of time where-

as 2 of them died at larval-pupal moult. The morphology and longevity of 6th instar larvae, however, remained unchanged. Pupation was normal and all the pupae successfully transformed into adults. The adult emergence was 11 percent less as compared to control (Table - 5). No morphological abnormality was noticed in the adults. When 6 affected females were separately paired with normal males of corresponding age the average number of eggs laid by each female was 560.16 eggs less than that of control. Further, the egg hatching was also reduced by 6.58 percent as compared to control (Table - 6). These observations on fecundity ($t = 4.17$, $p<0.05$) and fertility ($t = 4.61$, $p<0.05$) werestatistically significant.

In the third test each of the 100 larvae was fed 2.0 ug β-ecdysone. The ingestion of this dose resulted in the death of 7 larvae during the 5th instar. However, the morphology and longevity of the surviving larvae were unaffected. But at the next larval-larval moult 13 larvae suffered mortality. The morphology and longevity of the survived 6th stage larvae remained unchanged. Thereafter, 4 larvae died during the 6th instar. The remaining larvae pupated with the exception of 4 which died at larval-pupal moult. However, pupation was normal and all the pupae metamorphosed to normal adults. The adult emergence was 18 percent less as compared to control (Table - 5). The 6 affected females were mated with normal

males of corresponding age. Each female, on average, laid 967.83 eggs less than that of control. The percentage of egg hatching was also reduced by 3.35 percent as compared to control (Table - 6). These observations on fecundity ($t = 6.31$, $p<0.05$) and fertility ($t = 5.21$, $p<0.05$) were statistically significant.

In the fourth test, 100 larvae were allowed to ingest 4.0 ug β-ecdysone per larva. Then 2 larvae died soon after ingestion of this dose and 5 larvae died later in this instar (Table - 5). The morphology and longevity of the surviving larvae were normal. At the next larval moulting 2 larvae died. The larvae moulted to 6th instar were morphologically normal but their longevity was shortened by 8-10 hours. Again 6 larvae died during the 6th instar whereas another 6 larvae died at larval-pupal moult. Remaining larvae perfectly moulted to normal pupae which successfully metamorphosed to adults. Consequently adult emergence was reduced by 22 percent as compared to control. There were no abnormal adults. From this stock 6 affected females were mated separately with normal males of corresponding age. Each of the affected females, on average laid 1326.5 eggs less than control females (normal). The egg hatching was also reduced by 11.29 percent as compared to control (Table - 6). The reduction in fecundity ($t = 7.30$, $p<0.05$) and fertility ($t = 7.15$, $p<0.05$) was statistically significant.

In the fifth group of 100 test larvae, each larva was allowed to ingest 6.0 ug ecdysone. Soon after ingestion of this dose 8 larvae died and 2 larvae died later. However, the morphology of the larvae was unchanged but the larval longevity was abbreviated by 10-15 hours. The remaining larvae then moulted to 6th instar but 3 of them died at moulting. The larvae of 6th instar were morphologically normal. However, the longevity of this instar was shortened by 15-20 hours. Again 8 larvae died during the 6th instar whereas 7 larvae suffered mortality at larval-pupal moult. The remaining larvae pupated. There were 70 normal and 2 abnormal pupae. Out of these 4 normal and 2 abnromal pupae failed to metamorphose to adults. The abnormal pupae had larval head and abdominal appendages. Adult emergence was reduced by 34 percent as compared to control (Table - 5). All the adults were, however, normal. Further, 6 affected females were paired separately with normal males of corresponding age for observation on fecundity and fertility. Each of the affected females, on average, laid 1657.66 eggs less than that of control. The egg hatching was also reduced by 32.92 percent as compared to control. These changes on fecundity ($t = 8.93$, $p<0.05$) and fertility ($t = 8.28$, $p<0.05$) were statistically highly significant.

(D) Effect of ingestion of different doses of β-ecdysone on 6th instar larvae of Spodoptera litura:

From the stock culture 6th instar larvae, 24 hours old, were sorted out and then starved overnight. They were also fed on the known size (5 x 5 cm) of castor leaves sprayed with each of 0.5, 1.0, 2.0, 4.0 and 6.0 ug β-ecdysone as mentioned before. Each dose was tested against 100 larvae. The pieces of castor leaves treated with 1.0 ul acetone only served as food of control insects.

Thus in the first set of 100 test larvae, 0.5 ug β-ecdysone was fed to each larva. Then 4 larvae died during the larval duration. The ingestion caused no effect either on morphology or longevity of the larvae. The larvae normally moulted to pupal stage but 5 of them died at larval-pupal moult. The pupae were normal and all of them successfully metamorphosed to normal adults. However, the adult emergence was reduced by 4 percent as compared to control due to death of 9 treated larvae (Table - 7). Further, 6 affected females were paired separately with normal males of corresponding age to observe fecundity and fertility. Each of the affected females, on average, laid 663.5 eggs less than that of control. Fertility of the eggs was also reduced by 11.41 percent as compared to control (Table - 8). The fall in fecundity ($t = 2.07$, $p>0.05$) and fertility ($t = 2.51$, $p>0.05$) was statistically insignificant.

In the second group of 100 test larvae, each larva was fed 1.0 ug β-ecdysone. Due to ingestion of this dose 4 larvae developed lethargic condition and subsequently died whereas 4 other normal larvae died later. There was no change in the morphology and longevity of the larvae. However, 6 of them died at larval-pupal moult and remaining larvae pupated. All pupae except 4 successfully transformed into adults. The adult emergence was, therefore, 13 percent less as compared to control (Table - 7). Further, 6 affected females were paired separately with normal males of corresponding age for observing fecundity and fertility. On average, each of the affected females laid 819.33 eggs less than that of control. The egg hatching was also reduced by 3.12 percent as compared to control (Table - 8). These observations on fecundity ($t = 6.93$, $p<0.05$) and fertility ($t = 6.04$, $p<0.05$) were statistically significant.

In the third group of 100 test larvae, each larva was allowed to ingest 2.0 ug β-ecdysone. Due to ingestion 7 larvae died immediately whereas 5 larvae developed lethargic conditions and died a little later. The morphology and longevity of the larvae remained unaffected. Then 4 larvae attempted to moult to extra-instar stage but failed and died in the process. These larvae showed incomplete moulting and ecdysis was confined to thoracic regions only. Out of the remaining larvae, 8 died at larval-pupal moult and others pupated. But there were 7 normal

and 3 abnormal pupae which could not metamorphose to adults. The abnormal pupae had larval head and abdominal appendages. The adult emergence was reduced by 29 percent as compared to control (Table - 7). Thereafter, 6 females emerged from the treated larvae were mated separately with equal number of normal males of corresponding age. These females, on average, laid 1252.0 eggs less than that of control. The egg hatching was also reduced by 9.58 percent as compared to control (Table - 8). These observations were statistically highly significant (for fecundity $t = 8.74$, $p<0.05$ and for fertility, $t = 8.43$, $p<0.05$).

Further, in the fourth group of 100 test larvae, each larva was fed 4.0 ug β-ecdysone. Then, 10 larvae developed acute lethargic conditions half an hour after ingestion and ultimately died and 5 larvae died later. The morphology of the larvae remained unaffected. However, the ingestion of this dose shortened the larval duration by 20-24 hours. There was an attempt by 6 larvae to moult to extra-instar stage. As a result of this 5 of them died in the process but 1 of them successfully transformed into supernumerary larva (Table - 7) which after sometime also died. Further, 14 larvae died in the process of larval-pupal moulting. There was no emergence in 11 normal and 2 abnormal pupae. The adult emergence was thus reduced by 43 percent as compared to control (Table - 7). All the adults were normal. The fecundity of the affected females

suffered significantly ($t = 4.98$, $p < 0.05$) and each female, on average, laid 1710.66 eggs less as compared to control (Table - 8). The egg hatching was also significantly reduced by 31.89 percent as compared to control ($t = 6.10$, $p<0.05$).

In the fifth group of 100 test larvae, each larva was allowed to ingest 6.0 ug β-ecdysone. Then 15 larvae developed acute lethargic conditions soon after ingestion and ultimately died. Another 5 larvae died in the later course of larval duration. The morphology of the larvae showed no adverse effect. However, larval longevity was abbreviated by 34-36 hours. Again there was an attempt by 15 larvae to moult to extra instars. Although 10 of them died in the process, the remaining 5 larvae successfully transformed into supernumerary larvae (Table - 7) (Average weight = 0.95 gm; length = 4.88 cm, breadth = 0.75 cm as compared to normal 6th instar larva; average weight = 0.635 gm, length = 4.30 cm and breadth = 0.60 cm) which also died shortly afterwards. Further, 15 larvae died at larval-pupal moult showing incomplete ecdysis. There were 47 normal and 3 abnormal pupae. There was no emergence in 12 normal and 3 abnormal pupae. The malformed pupae had larval head and abdominal appendages. The adult emergence was 60 percent less as compared to control (Table - 7). Each of the affected females, on average, laid 2226.83 eggs less than that of control showing highly significant fall in fecundity ($t = 6.55$, $p<0.05$). The egg hatching was also significantly reduced

by 60.33 percent (Table - 8) as compared to control ($t = 11.85$, $p < 0.05$).

(E) Effect of injection of different doses of 'Triol' on 5th instar larvae of Spodoptera litura:

Each larva of 5th instar of S. litura was injected with one of the selected doses (0.5, 1.0, 2.0, 4.0 and 6.0 ug) of 'Triol'. Similarly larvae were injected with 1.0 ul acetone per larva to serve as parallel control.

In the first group of 100 test larvae, 0.5 ug 'Triol' was injected into each larva. It resulted in the death of 4 larvae during this instar and 7 larvae died at the next larval moulting. However, the morphology and longevity of 5th instar larvae remained unchanged. The larvae of 6th instar did not show any abnormality and 5 of them died at different intervals of time whereas 3 larvae died at larval-pupal moult. There was no abnormal formation in pupae. However, 15 pupae although normal in appearance failed to metamorphose to adults. The adult emergence was reduced by 24 percent as compared to control (Table - 9). Further, 6 affected females were individually mated with normal males of corresponding age for observations on fecundity and fertility. Each of the affected females, on average, laid 134.17 eggs less than that of control. Fertility of the eggs was, however, 0.22 percent more than that of control (Table - 10). The data on fecundity ($t = 1.52$, $p > 0.05$) and

fertility ($t = 1.50$, $p>0.05$) were insignificant.

In another experiment 100 test larvae of 5th instar were injected with 1.0 ug 'Triol' per larva. It was followed by the death of 6 larvae during the same instar. Further, 4 larvae died at larval-larval moult to 6th instar, 6 larvae died during 6th instar and 5 larvae died at larval-pupal moult. The morphology and longevity of either instar remained unchanged All pupae were normal. However, 27 pupae could not metamorphose to adults. The adult emergence was reduced by 38 percent as compared to control (Table - 9). Each affected female, on average, laid 600.67 eggs less than that of control. Fertility of the eggs was only slightly reduced (0.34 percent) as compared to control (Table - 10). These changes in fecundity ($t = 2.92$, $p<0.05$) and fertility ($t = 2.96$, $p<0.05$) were statistically significant.

In the third experiment each of 100 test larvae of 5th instar was injected with 2.0 ug 'Triol'. Mortality was observed at all stages of larval duration. Then, 6 larvae died at 5th instar whereas 3 larvae died at larval-larval moult. Further, 7 larvae of 6th instar also suffered mortality and another 9 larvae died at larval-pupal moult. The morphology and longevity of both 5th as well as 6th instar larvae were unaffected. Pupation was normal but 37 pupae failed to metamorphose to adults. The adult emergence was reduced by 52 percent as

compared to control (Table - 9). As regards the fecundity of the females so emerged (affected females) each female, on average, laid 966.0 eggs less than that of control. However, fertility of the eggs was reduced by .01 percent only (Table-10). These data on fecunility ($t = 3.72$, $p<0.05$) and fertility ($t = 3.72$, $p<0.05$) were statistically significant.

In the fourth set of 100 test larvae of 5th instar each larva was injected with 4.0 ug 'Triol'. Then 5 larvae died within 24 hours after injection and 4 larvae died at larval-larval moult. No change was observed either in morphology or longevity of the larvae. The 6th instar larvae, moulted from 5th instar, were also normal but 8 of them died at different intervals of time and 11 of them could not survive at larval-pupal moult. However, all the pupae were normal. But 41 normal pupae failed to metamorphose to adults. The loss after adult emergence was 59 percent as compared to control (Table - 9). When 6 females, emerged from the treated larvae, were paired individually with normal males of corresponding age each of these females, on average, laid 1167.84 eggs less than that of control and fertility of the females was reduced by 6.45 percent as compared to control (Table -10). The fall in fecundity ($t = 4.80$, $p<0.05$) and fertility ($t = 4.81$, $p<0.05$) was statistically significant.

In the 5th set of experiment 100 test larvae were injected with 6.0 ug 'Triol' per larva. It leads to the death of 2 larvae within an hour after injection whereas 5 larvae died within the next 24 hours. Though the morphology of the larvae remained unaffected, the longevity was, however, abbreviated by 16-35 hours. Further, 11 larvae of the 5th instar suffered mortality at moulting to 6th instar. There was no change in the morphology and longevity of 6th instar but 9 larvae died during this instar whereas 13 of them died at larval-pupal moult. Although all the pupae were normal, 48 pupae failed to metamorphose to adults. Finally, adult emergence was reduced by 78 percent as compared to control (Table - 9). Further, out of 6 females which were observed for fecundity, only one female laid eggs and the total number of these eggs was only 76. Out of these 45 eggs were fertile.

(F) Effect of injection of different doses of 'Triol' on 6th instar larvae of Spodoptera litura:

From the stock culture, 24 hour old 6th instar larvae of S. litura were sorted out and then injected with different doses (0.5, 1.0, 2.0, 4.0 and 6.0 ug per larva) of 'Triol' in the same way as in the 5th instar larvae. The larvae injected with 1.0 ul acetone only served as parallel control. Each dose was tested against 100 larvae.

In the first set of 100 test larvae, each larva was injected with 0.5 ug 'Triol'. Then 9 larvae died during this larval instar whereas 7 larvae died at larval-pupal moulting. The remaining larvae pupated normally and all except 33 pupae metamorphosed to normal adults. The adult emergence was, however, reduced by 37 percent as compared to control (Table-11). Each female emerged from treated larvae, on average, laid 432.33 eggs less than that of control and this drop was statistically significant ($t = 3.84$, $p<0.05$). Reduction in fertility was only 0.55 percent as compared to control (Table - 12) which was also statistically significant ($t = 3.93$, $p<0.05$).

In the second group of 100 larvae, 1.0 ug 'Triol' was injected to each larva. Consequently, 9 larvae died before larval-pupal moult and 18 larvae at larval-pupal moult. There was no change either in the larval morphology or longevity. The surviving larvae normally pupated and all except 38 pupae metamorphosed to equally normal adults. The emergence of adults was, however, 53 percent less than that of control (Table - 11). As regards the fecundity of females which emerged from treated larvae it was found that each of these females, on average, laid 901.66 eggs less than that of control. Reduction in fertility was 3.4 percent as compared to control (Table - 12). The observation on fecundity ($t = 5.33$, $p<0.05$) and fertility ($t = 5.27$, $p<0.05$) were statistically significant.

In the third batch of 100 test larvae each larva was injected with 2.0 ug 'Triol'. It resulted in the death of 13 larvae before pupal moulting whereas 22 larvae died during the moulting. Although the morphology of the larvae remained unaffected, the longevity of the larvae was shortened by 15-25 hours. Pupae were normal and all except 53 pupae successfully metamorphosed to adults which were also normal but their emergence was reduced by 76 percent as compared to control (Table-11). For observations on fecundity and fertility when the affected females were paired with normal males of corresponding age, each of the affected females, on average, laid 1103.5 eggs less than that of control. Fertility was, however, reduced only by 4.18 percent (Table - 12). The fall in fecundity ($t = 6.74$, $p<0.05$) and fertility was highly significant ($t = 6.91$, $p<0.05$).

In the fourth group of 100 larvae, each larva was injected with 4.0 ug 'Triol'. Among the treated larvae 17 developed lethargic conditions and died in due course of time whereas 21 larvae died at larval-pupal moult. However, morphology of the larvae was not affect but longevity was shortened by 20-35 hours. All the pupae were normal but none of them could metamorphose to adults (Table - 11).

In the fifth batch of 100 test larvae 6.0 ug 'Triol' was injected to each larva. Then 19 larvae developed acute lethargic conditions and ultimately died whereas 28 larvae

suffered mortality at larval-pupal moult. Though morphology of the larvae remained unaffected, their longevity was abbreviated by 30-40 hours. All the pupae were normal but none of them could metamorphose to adults (Table - 11).

(6) Effect of ingestion of different doses of 'Triol' on 5th instar larvae of Spodoptera litura:

Fifth instar larvae of S. litura (24 hour old) which were starved overnight, were individually fed with different doses of 'Triol' (0.5, 1.0, 2.0, 4.0, 6.0 ug/larva) by applying them on the known size of castor leaves (5 x 5 cm). Each dose was tested against 100 larvae. The control larvae were fed with 1.0 ul acetone/larva only.

In the first batch of 100 larvae, each larva ingested 0.5 ug 'Triol'. Then, 6 larvae died during the 5th instar and 5 larvae suffered mortality at next larval moult. It was followed by the death of 7 larvae in the 6th instar and the same number at larval-pupal moult. However, the longevity and morphology of the larvae were not affected either during the 5th or 6th instar. Pupal formation was normal and all the pupae successfully metamorphosed to adults. The adult emergence was reduced by 7 percent as compared to that of control (Table - 13). Following emergence each of the affected females, on average, laid 26.16 eggs less than that of control. Fertility of these females was reduced by 7.42 percent as compared to

control (Table - 14). The fall in fecundity ($t = 0.94$, $p>0.05$) and fertility ($t = 1.98$, $p>0.05$) were statistically insignificant.

In the second set of 100 larvae, each larva was allowed to ingest 1.0 ug 'Triol'. It caused mortality of 9 larvae during the same instar and 6 larvae at larval-larval moult. Further, 9 larvae died during the 6th larval instar and 4 larvae suffered mortality at larval-pupal moult. The morphology and longevity of both 5th and 6th instar larvae were unaffected. All the pupae were normal and these metamorphosed to normal adults. The adult emergence was reduced by 10 percent as compared to control (Table - 13). It was further found that each of the emerged females, on average, laid 66.66 eggs less than that of control. Reduction in fertility was 12.65 percent (Table - 14). The fall in fecundity ($t = 1.55$, $p>0.05$) was insignificant but it was significant in case of fertility ($t = 3.04$, $p<0.05$).

In the third batch of 100 larvae of 5th instar each larva was fed 2.0 ug 'Triol'. Following this 9 larvae died during the same larval stage and 9 larvae died at the next larval-larval moulting. However, the morphology and longevity of the 5th instar larvae were not affected. Further, 12 larvae died during the 6th instar and 9 larvae could not survive during the larval-pupal moulting. The morphology and longevity of the 6th instar also remained unaffected. All the pupae were

normal and metamorphosed to normal adults. However, adult emergence was reduced by 21 percent as compared to control (Table - 13). When the affected females (emerged from the treated larvae) were paired with normal males of corresponding age each female, on average, laid 145.16 eggs less than control which was statistically insignificant ($t = 2.12$, $p > 0.05$). Fertility was also reduced by 21.68 percent (Table - 14) as compared to that of control and this drop in fertility was significant ($t = 4.24$, $p < 0.05$).

In the fourth group of 100 larvae each larva was allowed to ingest 4.0 ug 'Triol'. This resulted in the death of 12 larvae during the same stage and 13 larvae at next larval-larval moulting. The morphology and longevity of these larvae were unaffected. The remaining larvae then moulted to 6th instar in which 10 larvae suffered mortality and 9 larvae died at larval-pupal moulting. However, no change either in morphology or longevity of this instar was seen. Among the pupae 8 were abnormal with larval head and abdominal appendages. All the abnormal and 5 normal pupae failed to metamorphose to adults. As a result of larval mortality and absence of emergence in pupae adult emergence was reduced by 39 percent as compared to control (Table - 13). As regards fecundity of the affected females each female, on average, laid 201.9 eggs less than that of control which was statistically significant ($t = 2.62$,

$p<0.05$). Fertility was reduced by 34.10 percent (Table - 14) and this drop was also significant ($t = 5.51$, $p<0.05$).

In the fifth batch of 100 larvae each larva was allowed to ingest 6.0 ug 'Triol'. Following this 13 larvae died during the 5th instar whereas 17 larvae suffered mortality at next moulting. Though the morphology of 5th instar larvae remained unchanged, the longevity of these larvae was abbreviated by 12-20 hours. Among the 6th instar larvae, 9 larvae suffered mortality at various intervals of time and 8 of them died at larval-pupal change. However, the morphology and longevity of the 6th instar larvae were not affected. Among the pupae 13 were abnormal with larval head and abdominal appendages. All the abnormal and all normal pupae could not metamorphose to adults. The adult emergence was thus reduced by 53 percent as compared to that of control (Table - 13). Further, the fecundity of the affected females significantly dropped ($t = 2.64$, $p<0.05$) as each female, on average, laid 209.0 eggs less as compared to that of control. Fertility was also reduced by 50.58 percent (Table - 14) which was statistically highly significant ($t = 5.70$, $p<0.05$).

(H) Effect of ingestion of different doses of 'Triol' on 6th instar larvae of Spodoptera litura:

Like the treatment by feeding different doses of 'Triol' to 5th instar larvae of S. litura, ingestion of these

doses of 'Triol' was allowed to 6th instar larvae (24 hour old and starved overnight). Each dose was fed to 100 larvae. The larvae fed with 1.0 ul acetone only served as parallel control.

In the first set, 100 test larvae were fed with 0.5 ug 'Triol' per larva. Following the ingestion 10 larvae died within 36 hours whereas 8 larvae suffered mortality at larval-pupal moult. However, the morphology and longevity of the larvae were unaffected. Among the pupae, 7 pupae were malformed having larval head and abdominal appendages. All the pupae, except malformed, transformed into normal adults. The emergence of adults was, however, reduced by 7 percent as compared to control (Table - 15). Further, each affected female, on average, laid 47.50 eggs less than that of control (Table - 16) showing insignificant drop ($t = 1.31$, $p>0.05$). Fertility was reduced by 11.47 percent as compared to control. This change was also statistically insignificant ($t = 2.36$, $p>0.05$).

In the second group 1.0 ug 'Triol' was fed to each of the 100 test larvae. Thereafter, 10 larvae died at different intervals of time but 9 larvae could not survive at larval-pupal moulting. Among the pupae 5 pupae were malformed. These malformed pupae and 5 normal pupae could not metamorphose to adults. However, the emerged adults were normal. The morphology and longevity of the 6th instar larvae were normal. The adult

emergence was reduced by 11 percent as compared to control (Table - 15). It was further recorded that each of the affected females, on average, laid 80.17 eggs less than that of control showing insignificant afall ($t = 2.02$, $p>0.05$). Fertility was also reduced by 24.99 percent as compared to control (Table-16) and this drop was statistically significant ($t = 4.13$, $p<0.05$).

In the third batch 2.0 ug 'Triol' was fed to each of the 100 test larvae. It was followed by the death of 8 larvae during the same stage and 14 of them died at larval-pupalmo moulting. The morphology and longevity of the 6th instar larvae were unaffected. There were 10 abnormal and 9 normal pupae. All the abnormal and 9 normal pupae failed to metamorphose to adults. All the emerged adults were normal. The adult emergence was reduced by 23 percent as compared to control (Table - 15). Further, each of the affected females, on average, laid 123.0 eggs short of that of control which was statistically insignificant drop ($t = 2.50$, $p>0.05$). Fertility of the eggs was dropped by 36.31 percent as compared to control (Table - 16) and it was statistically significant ($t = 3.75$, $p<0.05$).

In the fourth group of 100 test larvae each larva was fed with 4.0 ug 'Triol'. It caused lethargic conditions in the larvae and 13 of them ultimately died. Then 12 larvae died at larval-pupal moulting. Though the morphology of the larvae remained unchanged, the longevity was shortened by 15-20 hours.

After pupation 15 pupae were seen as abnormal. The abnormal pupae and 17 normal pupae failed to metamorphose to adults. The emergence was reduced by 39 percent as compared to control (Table - 15). The fecundity of the affected females was significantly dropped ($t = 2.98$, $p<0.05$) as each of the affected females, on average, laid 174.83 eggs less than that of control. Fertility was also reduced by 59.47 percent as compared to control (Table - 16) and this drop was statistically highly significant ($t = 6.01$, $p<0.05$).

In the fifth group of 100 test larvae each larva was fed with 6.0 ug 'Triol'. Then the larvae developed acute lethargic conditions and 20 of them died within 10 hours after ingestion. Further, 16 larvae died at larval-pupal moulting. However, the morphology of the larvae was not affected but the longevity was shortened by 15-25 hours as compared to control. Among the pupae 17 were abnormal pupae. All such pupae and 23 normal pupae failed to metamorphose to adults. The adult emergence was reduced by 58 percent as compared to control (Table - 15). Each of the affected females, on average, laid 189.83 eggs less than that of control significantly reducing the fecundity ($t = 2.61$, $p<0.05$). As regards fertility, it was also significantly dropped by 78.76 percent (Table - 16) as compared to that of control ($t = 6.21$, $p<0.05$).

Table - 1: Showing larval and pupal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of β-ecdysone to the 5th instar larvae of Spodoptera litura (Each dose was tested against 100 larvae).

Dose/larva (ug)	Larval Mortality (Number)				Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss upto emergence (%)
	5th instar	At next moult	6th instar	At larval-pupal moult	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	2	-	2	1	95	-	-	-	95	-	5
0.5	2	3	3	2	90	-	-	-	90	-	10
1.0	3	3	4	2	88	-	-	-	88	-	12
2.0	5	5	4	4	82	-	-	-	82	-	18
4.0	8	8	10	9	65	-	-	-	65	-	35
6.0	12	8	14	10	53	-	3	-	53	-	47

Average of 5 replicates

Table - 2: Showing fecundity and fertility of females of Spodoptera litura emerged from 5th instar larvae injected with different doses of β-ecdysone.

Dose/larva (ug)	No. of eggs laid (mean value) ± S.E.	No. of eggs hatched (mean value) ± S.E.	% hatching
Control	2408.66 ± 29.43	2252.50 ±19.72	93.51
0.5	2272.50 ± 23.19	2080.00* ± 35.66	91.52
1.0	2094.50 ± 46.34	1678.66* ± 38.23	82.91
2.0	1618.83* ± 9.01	1038.33 ±209.32	64.14
4.0	916.16 ±184.41	664.83 ±138.36	72.56
6.0	720.16* ± 16.02	237.16* ± 52.60	32.91

* Significant at 5% level

Table - 3: Showing larval mortality, supernumerary larvae, pupal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of β-ecdysone to the 6th instar larvae of Spodoptera litura (Each dose was tested against 100 larvae).

Dose/ larva (ug)	Larval Mortality (Number)				Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss up to emergence (%)
	6th instar	At larval-pupal moult	At moulting to extra instar	Supernumerary larvae	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	6	4	-	-	90	-	-	-	90	-	10
0.5	6	6	-	-	88	-	-	-	88	-	12
1.0	8	6	-	-	86	-	4	-	82	-	18
2.0	13	8	-	-	79	-	5	-	74	-	26
4.0	13	10	8	-	67	2	7	2	60	-	40
6.0	17	13	10	2	55	3	10	3	45	-	55

Average of 5 replicates

Table - 4: Showing fecundity and fertility of females of Spodoptera litura emerged from 6th instar larvae injected with different doses of β-ecdysone.

Dose/larva (ug)	No. of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	2035.83 ± 3[illegible].70	1838.38 ± 32.80	90.30
0.5	1889.83 ± 89.39	1633.33* ± 32.65	86.00
1.0	1545.16* ± 18.38	1236.16* ± 40.77	80.00
2.0	1145.33* ± 22.18	972.00* ± 15.72	84.86
4.0	891.83* ±187.82	382.00* ± 81.94	42.66
6.0	382.83* ±121.38	77.16* ± 38.29	20.15

*Significant at 5% level.

Table - 5: Showing larval and pupal mortality, adult emergence and total loss up to adult emergence following the ingestion of different doses of β-ecdysone by the 5th instar larvae of *Spodoptera litura* (Each dose was tested against 100 larvae).

Dose/ larva (ug)	Larval Mortality (Number)				Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss upto emergence (%)
	5th instar	At next moult	6th instar	At larval-pupal moult	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	-	-	-	-	100	-	-	-	100	-	0
0.5	5	-	2	1	92	-	-	-	92	-	8
1.0	5	1	3	2	89	-	-	-	89	-	11
2.0	7	3	4	4	82	-	-	-	82	-	18
4.0	8	2	6	6	78	-	-	-	78	-	22
6.0	10	3	8	7	70	2	4	2	66	-	34

Average of 5 replicates

Table - 6: Showing fecundity and fertility of females of Spodoptera litura emerged from 5th instar larvae ingesting different doses of β-ecdysone.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	2738.16 ± 39.22	2461.33 ± 45.14	89.88
0.5	2260.66* ± 19.63	2061.16* ± 21.63	91.17
1.0	2178.00* ± 39.66	1814.33* ± 29.21	83.30
2.0	1780.33* ± 19.58	1540.66* ± 37.74	86.53
4.0	1411.66* ± 21.77	1109.50* ± 19.50	78.59
6.0	1080.50* ± 11.61	615.50* ± 20.68	56.96

* Significant at 5% level.

Table - 7: Showing larval mortality, supernumerary larvae, pupal mortality, adult emergence and total loss up to adult emergence following the ingestion of different doses of β-ecdysone by the 6th instar larvae of Spodoptera litura (Each dose was tested against 100 larvae).

Dose/ larva (ug)	Larval Mortality (Number)				Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss upto emergence(%)
	6th instar	At larval-pupal moult	At moulting to extra-instar	Super-numerary larvae	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	4	1	-	-	95	-	-	-	95	-	5
0.5	4	5	-	-	91	-	-	-	91	-	9
1.0	8	6	-	-	86	-	4	-	82	-	18
2.0	12	8	4	-	73	3	7	3	66	-	34
4.0	15	14	5	1	63	2	11	2	52	-	48
6.0	20	15	10	5	47	3	12	3	35	-	65

Average of 5 replicates

Table - 8: Showing fecundity and fertility of females of *Spodoptera litura* emerged from 6th instar larvae ingesting different doses of β-ecdysone.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	2475.16 ± 13.71	2154.66 ± 25.52	87.05
0.5	1811.66 ±363.84	1370.50 ±277.77	75.64
1.0	1655.83* ± 28.03	1389.83* ± 25.80	83.93
2.0	1223.16* ± 26.37	947.66* ± 16.00	77.47
4.0	764.50* ±154.83	424.00* ± 88.24	55.46
6.0	248.83* ±113.39	66.50* ± 10.91	26.72

* Significant at 5% level

Table - 9: Showing larval and pupal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of 'Triol' to the 5th instar larvae of Spodoptera litura (Each dose was tested against 100 larvae).

Dose/ larva (ug)	Larval Mortality (Number)				Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss upto emergence (%)
	5th instar	At next moult	6th instar	At larval-pupal moult	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	-	3	2	5	90	-	-	-	90	-	10
0.5	4	7	5	3	81	-	15	-	66	-	34
1.0	6	4	6	5	79	-	27	-	52	-	48
2.0	6	3	7	9	75	-	37	-	38	-	62
4.0	5	4	8	11	72	-	41	-	31	-	69
6.0	7	11	9	13	60	-	48	-	12	-	88

Average of 5 replicates

Table - 10: Showing fecundity and fertility of females of Spodoptera litura emerged from 5th instar larvae injected with different doses of 'Triol'.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	1290.0 ± 93.81	1270.16 ± 97.71	98.46
0.5	[illegible] ± [illegible]	1140.66 ± 42.28	[illegible]
1.0	789.33* ± 44.65	774.5* ± 40.20	98.12
2.0	324.0* ± 71.48	319.0* ± 69.34	98.45
4.0	102.16* ± 27.34	94.0* ± 26.62	92.01
6.0	-	-	-

* Significant at 5% level

Table - 11: Showing larval and pupal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of 'Triol' to the 6th instar larvae of Spodoptera litura (Each dose was tested against 100 larvae).

Dose/larva (ug)	Larval Mortality (Number)		Pupation (Number)		Pupal Mortality (Number		Adult Emergence (Number)		Total loss up to emergence (%)
	6th instar	At larval-pupal moult	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	6	6	88	-	-	-	88	-	12
0.5	9	7	84	-	33	-	51	-	49
1.0	9	18	73	-	38	35	35	-	65
2.0	13	22	65	-	53	-	12	-	88
4.0	17	21	62	-	62	-	Nil	-	100
6.0	19	28	53	-	53	-	Nil	-	100

Average of 5 replicates

Table - 12: Showing fecundity and fertility of females of Spodoptera litura emerged from 6th instar larvae injected with different doses of 'Triol'.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	1253.66 ± 26.50	1246.33 ± 25.54	99.41
0.5	821.33* ± 45.26	812.0* ± 43.29	98.86
1.0	452.00* ± 42.55	434.16* ± 46.03	96.01
2.0	150.16* ± 32.96	143.0* ± 30.23	95.23
4.0	-	-	-
6.0	-	-	-

*Significant at 5% level

Table - 13: Showing larval and pupal mortality, adult emergence and total loss up to adult emergence following the ingestion of different doses of 'Triol' by the 5th instar larvae of Spodoptera litura (Each dose was tested against 100 larvae).

Dose/ larva (ug)	Larval Mortality (Number)				Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss upto emergence (%)
	5th instar	At next moult	6th instar	At larval-pupal moult	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	4	3	6	5	82	-	-	-	82	-	18
0.5	6	5	7	7	75	-	-	-	75	-	25
1.0	9	6	9	4	72	-	-	-	72	-	28
2.0	9	9	12	9	61	-	-	-	61	-	39
4.0	12	13	10	9	51	5	8	5	43	-	57
6.0	13	17	9	8	42	11	13	11	29	-	71

Average of 5 replicates

Table - 14: Showing fecundity and fertility of females of Spodoptera litura emerged from 5th instar larvae ingesting different doses of 'Triol'.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	1114.66 ± 37.89	1058.00 ± 21.87	94.91
0.5	1088.50 ± 33.41	952.33 ± 43.85	87.49
1.0	1048.00 ± 29.33	862.16* ± 29.77	82.26
2.0	969.50 ± 41.00	710.00* ± 25.52	73.23
4.0	913.66* ± 33.52	555.66* ± 18.58	60.81
6.0	905.66* ± 35.37	401.50* ± 27.48	44.33

* Significant at 5% level

Table - 15: Showing larval and pupal mortality, adult emergence and total loss up to adult emergence following the ingestion of different doses of 'Triol' by th 6th instar larvae of Spodoptera litura (Each dose was tested against 100 larvae).

Dose/larva (ug)	Larval Mortality (Number)		Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss up to emergence (%)
	6th instar	At larval-pupal moult	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	9	6	85	-	3	-	82	-	18
0.5	10	8	82	-	7	-	75	-	25
1.0	10	9	76	5	5	5	71	-	29
2.0	8	14	69	9	10	9	59	-	41
4.0	13	12	60	15	17	15	43	-	57
6.0	20	16	47	17	23	17	24	-	76

Average of 5 replicates

Table - 16: Showing fecundity and fertility of females of *Spodoptera litura* emerged from 6th instar larvae ingesting different doses of 'Triol'.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	1086.33 ± 25.25	1070.33 ± 21.05	98.52
0.5	1038.83 ± 41.86	904.33 ± 51.75	87.05
1.0	1006.16 ± 22.50	739.83* ± 35.24	73.53
2.0	963.33 ± 22.97	599.33* ± 60.89	62.21
4.0	911.50* ± 22.89	356.00* ± 27.32	39.05
6.0	896.50* ± 42.95	177.16* ± 59.46	19.76

* Significant at 5% level

Fig. 1 Formation of supernumerary larva in Spodoptera litura following the treatment with β-ecdysone.

A. Supernumerary larva

B. Normal 6th instar larva.

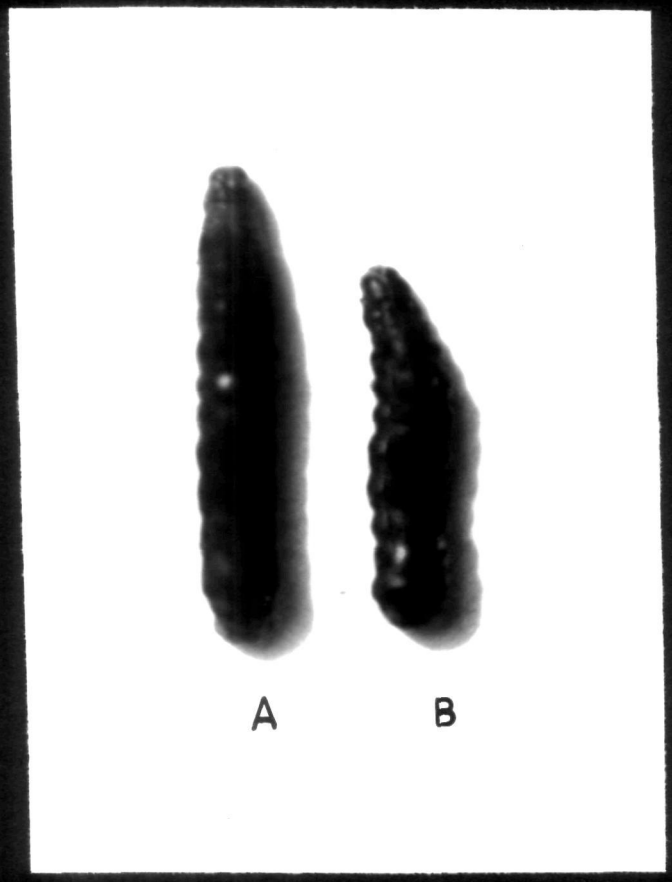

FIG. 1

2. Diacrisia obliqua:

(A) Effect of injection of different doses of α-ecdysone on 5th instar larvae of Diacrisia obliqua:

Doses : 0.5, 1.0, 2.0, 4.0 and 6.0 ug/larvae

Like the 5th instar larvae of S. litura those of D. obliqua were also injected with ecdysone. But in these larvae which were 24 hours old, doses of α-ecdysone were injected. However, the doses of α-ecdysone were similar to those of β-ecdysone. For parallel control these larvae were injected with 1.0 ul acetone only. For each dose 100 larvae were experimented.

In the first batch of test larvae, 0.5 ug α-ecdysone was injected to each larva. After this 10 larvae died during the 5th instar and 5 larvae could not survive during the next moult. The morphology and longevity of 5th instar larvae were unaffected. Following moulting 9 larvae of 6th instar died at different intervals of time whereas 3 larvae died at larval-pupal moult. The morphology and longevity of the 6th instar larvae were also unaffected. Pupae were normal and all but 4 transformed into normal adults. The adult emergence was 8 percent less as compared to control (Table - 17). Further, for observations on fecundity and fertility when 6 females emerged from the treated larvae were mated with normal males of corresponding age, it was found that each of the affected females, on average, laid 11.67 eggs less than that of control (Table - 18) which was statisti-

cally insignificant (t = 0.86, p>0.05). Fertility was reduced by 4.53 percent as compared to control and this was also statistically insignificant (t = 1.57, p>0.05).

In the second batch of test larvae, each larva was injected with 1.0 ug ∝-ecdysone (Table - 17). It was observed that 9 larvae died during the same stage. Among the remaining larvae, 6 larvae developed attenuated cuticle (Fig. 2, B) which could not be cast off at moulting to 6th instar resulting in the ultimate death of these larvae. The remaining larvae normally moulted to 6th instar. Normal and living larvae of 5th instar had no change in morphology and longevity. Then, 10 larvae died during the 6th instar and 2 larvae could not survive at larval-pupal moult. Further, 4 larval-pupal intermediates (Fig.2, P) were also formed which could not pupate and ultimately died. Among the pupae 4 normal (Fig.2, E) and 3 malformed pupae (Fig.2, M) failed to metamorphose to adults. The malformed pupae had diminished size and larval cuticle still intact with them. The adult emergence was reduced by 15 percent as compared to control (Table-17). The fecundity of each of affected females suffered insignificantly (t = 1.67, p>0.05) by laying 64.84 eggs less than that of control (Table-18). Reduction in fertility was 1.56 percent as compared to control which was statistically insignificant(t= 1.81, p>0.05).

In the third batch, each larva was injected with 2.0 ug ∝-ecdysone. Among the treated larvae 10 died at the same stage

whereas 5 died at moulting to 6th instar. Though the morphology of 5th instar larvae remained unchanged the larval longevity was shortened by 12-15 hours. However, the larvae of the 6th instar did not show any change in morphology and longevity. But 9 of them died at different intervals of time and 3 larvae could not surivive at larval-pupal moult. Moreover, 7 larval-pupal intermediates characterised with larval abdominal appendages and pupal body-shape (Fig. 2, P) were also formed. Among the pupae, 3 normal and 3 malformed pupae failed to metamorphose to adults. The adult emergence was reduced by 17 percent as compared to control. Among the adults, 13.33 percent (Table - 17) were abnormal with folded fore- and hind-wings (Fig. 4, a). The malformed adults could not continue and died on the very day of emergence. It was found that each of the affected females, on average, laid 109.50 eggs less than that of control. Fertility of the eggs was also reduced by 4.76 percent as compared to control (Table - 18). The data on fecundity ($t = 1.87$, $p > 0.05$) and fertility ($t = 2.33$, $p > 0.05$) were statistically insignificant.

In the fourth batch of test larvae, each larva was injected with 4.0 ug α-ecdysone. It was followed by the death of 12 larvae during this stage and 5 larvae at moulting to 6th instar. The morphology of the treated 5th instar larvae was not affected but the longevity was abbreviated by

20-30 hours. Among the 6th instar larvae, 8 larvae died within 20 hours after moulting and 4 larvae died at larval-pupal moult. Aside this, 5 larvae transformed to larval-pupal intermediates which died before pupation. Following pupation, 5 normal and 5 abnormal pupae could not metamorphose to adults. Finally, the loss of adult emergence was 21 percent as compared to control. Following emergence, 10.71 percent (Table - 17) adults were malformed (Fig. 4, a). Further, each of the normally emerged females from the treated larvae, on average, laid 181.84 ergs less than that of control showing statistically insignificant fall ($t = 2.33$, $p > 0.05$). Reduction in fertility of the females was 6.05 percent as compared to control (Table - 18). These data on fertility was, however, statistically significant ($t = 2.67$, $p < 0.05$).

In the fifth batch of test larvae, each larva was injected with 6.0 ug α-ecdysone. The injection caused the death of 13 larvae at various intervals of time during the instar whereas 5 larvae died at next larval-larval moult. The morphology of the treated larvae of 5th instar remained unaffected but the longevity was shortened by 25-36 hours. Among the 6th instar larvae 6 died soon after moulting from 5th instar whereas 6 died at larval-pupal moult. The larvae of the 6th instar in their attempt to pupate developed into 12 larval-pupal intermediates which could not pupate and ultimately died. The remaining

larvae normally pupated but 48 normal and 10 abnormal pupae were formed. Out of th se 7 normal and 10 abnormal pupae failed to metamorphose to adults. The adult emergence was reduced by 36 percent as compared to control. Among the emerged adults 26.83 percent (Table - 17) were malformed (Fig. 4a). It was also found that each of the affected females, on average, laid 155.67 eggs less than that of control. Reduction in fertility was 8.26 percent as compared to control (Table - 18). These observations on fecundity ($t = 2.03$, $p>0.05$) and fertility ($t = 2.34$, $p>0.05$) were statistically insignificant.

(B) Effect of injection of different doses of ∝-ecdysone on 6th instar larvae of Diacrisia obliqua:

Newly moulted 6th instar larvae (24 hours old) were sorted out from the stock culture and injected with doses of ∝-ecdysone similar to that of 5th instar (0.5, 1.0, 2.0, 4.0 and 6.0 ug/larva). The larvae injected with 1.0 ul aceton only served as parallel control. Each dose was tested against 100 larvae.

In the first group 100 test larvae, each larva was injected with 0.5 ug ∝-ecdysone. Then it was observed that 11 larvae died at the treated larval instar whereas 5 larvae could not survive at larval-pupal moult. The morphology and longevity of the surviving larvae remained unaffected and these larvae normally pupated. All pupae except 7 metamorphosed to normal

adults. The adult emergence was reduced by 7 percent as compared to control (Table - 19). As regards the fecundity of the affected females it was found that each of the affected females, on average, laid 65.50 eggs less than that of control (Table - 20). Fertility was also reduced by 1.04 percent as compared to control. The data on fecundity ($t = 1.53$, $p > 0.05$) and fertility ($t = 1.62$, $p > 0.05$) were statistically insignificant.

In the second group of 100 test larvae, each larva was injected with 1.0 ug α-ecdysone. This dose caused the death of 9 larvae at the same stage and 5 larvae at larval-pupal moult. Further, 5 larval-pupal intermediates (Fig. 2, C). were formed at larval-pupal moult which failed to pupate and ultimately died. There was no malformation in pupae but all except 9 pupae successfully transformed into adults. The adult emergence was reduced by 12 percent as compared to control. Further, 8.33 percent adults were malformed (Table - 19) with fore- and hind-wings underdeveloped (Fig. 4,b). The fecundity and fertility of the affected females were considerably dropped (Table - 20). Each of the affected females, on average, laid 66.17 eggs less than that of control whereas reduction in fertility was 3.64 percent. The reduction in fecundity ($t = 1.72$, $p > 0.05$) and fertility ($t = 2.17$, $p > 0.05$) was statistically insignificant.

In the third group of 100 test larvae, each larva was injected with 2.0 ug α-ecdysone. Then, 8 larvae died during

the 6th instar (treated) and 8 larvae died at larval-pupal moult. Further, 4 larval-pupal intermediates (Fig. 2, G) were also formed which died before pupation. However, pupation was normal and all except 8 pupae metamorphosed to adults. All emerged adults were normal. Finally the adult emergence was reduced by 12 percent as compared to control (Table - 19). Each of the affected females, on average, laid 87.17 eggs less than that of control. Fertility was reduced by 0.11 percent as compared to control (Table - 20). These observations on fecundity ($t = 2.31$, $p > 0.05$) and fertility ($t = 2.23$, $p > 0.05$) were statistically insignificant.

In the fourth group of 100 test larvae, each larva was injected with 4.0 ug α-ecdysone. Among the treated larvae 12 larvae developed into neotenic forms (Fig. 2, C) which ultimately died whereas 7 normal larvae died at larval-pupal moult. There were 8 larval-pupal intermediates (Fig. 2, C) which also died before emergence. There was no malformation in pupae but 8 pupae failed to metamorphose to adults. The adult emergence was reduced by 19 percent as compared to control. Among the adults 7.70 percent (Table - 19) were malformed with underdeveloped fore- and hind-wings (Fig. 4,b). Further, each of the affected females, on average, laid 171.17 eggs less than that of control. Fertility was also reduced by 0.66 percent as compared to control (Table - 20). These observations on fecundity

($t = 3.05$, $p<0.05$) and fertility ($t = 2.90$, $p<0.05$) were statistically significant.

Again, in the fifth group of 100 larvae, each larva was injected with 6.0 ug ∝-ecdysone. This dose caused 15 larvae to turn into extreme neotenic forms with their body covered with hairs (Fig. 2, D). These neotenic forms died before moulting to pupal stage whereas 6 treated larvae died at larval-pupal moult. Out of the remaining larvae, 6 larval-pupal intermediates were formed (Fig. 2, C) which also died. The rest of the 6th instar larvae transformed to normal pupae and all except 9 pupae (normal) successfully metamorphosed to adults. The adult emergence was reduced by 20 percent as compared to control. Among the adults, 14.06 percent (Table - 19) were malformed with underdeveloped fore- and hind-wings (Fig. 4,b). The fecundity of the affected females dropped by 269.67 eggs per female as compared to control. The reduction in fertility was 3.60 percent as compared to control (Table - 20). These observations on fecundity ($t = 2.11$, $p>0.05$) and fertility ($t = 2.38$, $p>0.05$) were statistically insignificant.

(C) <u>Effect of ingestion of different doses ∝-ecdysone on 5th instar larvae of Diacrisia obliqua</u>:

Twenty four hours old 5th instar larvae were starved overnight and then allowed to ingest selected doses of ∝-ecdysone. The larvae ingesting leaf pieces treated with 1.0 ul

acetone only served as parallel control. Each dose was tested against 100 larvae.

In the first group of 100 test larvae each larva was fed 0.5 ug ∝-ecdysone. Then, 10 larvae died during the 5th instar and 4 larvae died at next larval moulting. The morphology and longevity of the larvae were unaffected. The 6th instar larvae (moulted from 5th instar) also showed normal morphology and longevity but 4 of them died before pupation and 15 larvae died at larval-pupal moult. No larval-pupal intermediates or malformed pupae were observed. However, 4 normal pupae failed to metamorphose to adults. Although the adults were normal, there was 19 percent reduction in the adult emergence as compared to control (Table - 21). Further, it was found that each of the affected females, on average, laid 45.34 eggs less than that of control. There was 2.19 percent reduction in fertility also (Table - 22). The observations on fecundity ($t = 1.2$, $p>0.05$) and fertility ($t = 1.38$, $p>0.05$) were statistically insignificant.

In the second group of 100 test larvae, each larva was fed 1.0 ug ∝-ecdysone. The ingestion resulted in the death of 15 larvae during the same stage and 5 larvae at next larval moult. There was no change in the morphology and longevity of the larvae. Now, 6 of the 6th instar larvae also suffered mortality. Another 12 larvae died at larval-pupal moult. There

was no change in the morphology and longevity of the 6th instar. No larval-pupal intermediate or abnromal pupae were formed. All the pupae metamorphosed to normal adults. Reduction in the number of adults emerged was 28 percent as compared to control (Table - 21). Further, it was found that each of the affected females, on average, laid 157.34 eggs less than that of control. Fertility of the eggs was also reduced by 0.55 percent as compared to control (Table - 22). The observations on fecundity ($t = 2.25$, $p > 0.05$) and fertility ($t = 2.21$, $p > 0.05$) were statistically insignificant.

In the third group of 100 test larvae, each larva was fed 2.0 ug α-ecdysone. Out of these 17 died at the same stage and 6 larvae died at the next larval moult. The morphology and longevity of the larvae were unaffected. Then another 8 larvae died during 6th instar and 16 larvae at larval-pupal moult. The morphology and longevity of the 6th instar larvae also remained unchanged. No larval-pupal intermediates or malformed pupae were found. However, out of 53 normal pupae 12 pupae failed to metamorphose to adults. All the adults were normal. There was 41 percent reduction in the number of adults after emergence as compared to control (Table - 21). It was found that each of the affected females, on average, laid 143.67 eggs less than that of control which was statistically insignificant change ($t = 2.47$, $p > 0.05$). Fertility of the eggs was reduced by 1.65 percent as compared to control (Table - 22)

which was also statistically insignificant ($t = 2.54$, $p > 0.05$).

In the fourth group of 100 test larvae, each larva was fed 4.0 ug α-ecdysone. Out of these treated larvae 20 died during this instar and 5 larvae died at next larval moulting. The morphology and longevity of these larvae were not affected. Then 10 larvae of the succeding instar also died at different intervals of time but 19 of them died at larval-pupal moult. There was no formation of larval-pupal intermediate. The 6th instar larvae also maintained normal morphology and longevity. Pupation was normal but 9 pupae could not metamorphose to adults. There was 45 p rcent reduction in the number of adults (Table - 21). All the adults were normal. There was significant loss in fecundity as each of the affected females, on average, laid 225.34 eggs less than that of control ($t = 3.05$, $p < 0.05$). Fertility of the eggs was also reduced by 1.33 percent as compared to control (Table - 22) which was statistically significant fall ($t = 3.12$, $p < 0.05$).

In the fifth group of 100 test larvae, each larva was fed 6.0 ug α-ecdysone. This dose caused death of 22 larvae of the treated instar at various intervals of time but 8 of them died at next larval moult. No change either in morphology or longevity of these instar was noticed. Then 7 of the 6th instar larvae also died and 23 larvae died at larval-pupal moult. Pupation was normal but 11 pupae could not metamorphose

to adults. Consequently there was 53 percent reduction in the number of adults as compared to control (Table - 21). Among the adults 3.45 percent were malformed with folded fore- and hind wings (Fig. 4, e). Further, it was found that each of the affected females, on average, laid 220.17 eggs less than that of control. Fertility of eggs was also reduced by 2.27 percent as compared to control (Table - 22). These changes in fecundity ($t = 3.06$, $p<0.05$) and fertility ($t = 3.08$, $p<0.05$) were statistically significant.

(D) Effect of ingestion of different doses of ∝-ecdysone on 6th instar larvae of Diacrisia obliqua:

As mentioned before similar doses of ∝-ecdysone were fed to the 6th instar larvae of D. obliqua, which were 24 hours old and starved overnight before feeding the doses. Acetone treated leaves were fed to control insects.

In the first group of the 100 test larvae, each larva was fed 0.5 ug ∝-ecdysone. The ingestion of this dose caused death of 20 larvae during this instar at different intervals of time and 4 at larval-pupal moult. No larval-pupal intermediates or malformed pupae were noticed. Out of 76 normal pupae, only 1 pupa could not metamorphose to adult. All the adults formed were normal. The emergence of adults was reduced by 4 percent as compared to control (Table - 23). Further, it was found

that each of the affected females, on average, laid 86.83 eggs less than that of control. Fertility of the eggs was reduced only by 0.48 percent as compared to control (Table-24). The observations on fecundity ($t = 2.00$, $p > 0.05$) and fertility ($t = 2.24$, $p > 0.05$) were statistically insignificant.

In the second experiment each of the 100 test larvae was fed 1.0 ug α-ecdysone. Thus, 21 larvae of the treated instar died before pupation. Pupation was normal but 8 pupae could not transform into adults. All the adults were normal. In comparison to control the adults emergence was, however, reduced by 8 percent (Table - 23). Each of the affected females on average, laid 71.50 eggs less than that of control. Fertility of the eggs was also reduced by 5.36 percent as compared to control (Table - 24). The fall in fecundity ($t = 2.25$, $p > 0.05$) was insignificant but that of fertility ($t = 2.92$, $p < 0.05$) it was statistically significant.

In the third experiment each of the 100 test larvae was fed 2.0 ug α-ecdysone. The dose caused the death of 22 larvae during the same stage whereas 3 larvae died at larval-pupal moult. There was no change in the morphology and longevity of the larvae. No larval-pupal intermediates or malformed pupae were noticed. Pupation was also normal but 11 pupae failed to metamorphose to adults. Following emergence adults were normal. Up to adult emergence the loss of insects was 15 percent as

compared to control (Table - 23). Each of the affected females, on average, laid 128.50 eggs less than that of the control. Fertility of the eggs was also reduced by 1.43 percent as compared to control. The fall in fecundity ($t = 2.98$, $p<0.05$) and fertility ($t = 3.43$, $p<0.05$) was statistically significant.

In the fourth group of 100 test larvae, each larva was allowed to ingest 4.0 ug α-ecdysone. This dose caused the death of 30 larvae during the same stage and 6 larvae at the larval-pupal moult. The morphology and longevity of the treated instar was unaffected. There was no formation of larval-pupal intermediates. Pupation was also normal but 8 pupae could not metamorphose to adults. Though all the adults were normal their number, as compared to control, was reduced by 23 percent (Table - 23). The affected females, on average, laid 205.33 eggs less than that of the control. Fertility of the eggs was also reduced by 4.84 percent as compared to control (Table - 24). The observations on fecundity ($t = 3.07$, $p<0.05$) and fertility ($t = 3.91$, $p<0.05$) were statistically significant.

In the fifth group of 100 test larvae, each larva was allowed to ingest 6.0 ug α-ecdysone. Thus 32 larvae of the treated instar died following this ingestion at various intervals of time. Again 6 larvae died at larval-pupal moult. There was no change in the morphology and longevity of the larvae. No formation of larval-pupal intermediates was noticed.

All the pupae were normal but 11 of them could not metamorphose to adults. However, the number of adults was reduced by 28 percent as compared to the control and 25.5 percent (Table-23) were malformed with folded and underdeveloped fore- and hind-wings (Fig. 4, d). Each of the affected females, on average, laid 220.17 eggs less than that of control which was a significant fall ($t = 3.57$, $p<0.05$). The reduction in fertility was only 2.77 percent as compared to control (Table - 24) which was also statistically significant ($t = 4.00$, $p<0.05$).

(E) Effect of injection of different doses of 'Triol' on 5th instar larvae of Diacrisia oblicua:

For this experiment, 5th instar larvae (24 hours old) were injected with different doses of 'Triol'. For parallel control, each larva was injected with 1.0 ul acetone only. Each dose was tested against 100 larvae.

In the first experiment, each of the 100 larvae was injected with 0.5 ug 'Triol'. It led to the death of 9 larvae during the 5th instar larval duration whereas 9 larvae died at moulting from 5th to 6th instar. There was no change either in morphology or longevity. Among the 6th instar, 6 larvae died before pupation and 11 larvae at larval-pupal moult. The surviving larvae during the 6th instar had normal structure and longevity. Further, there was no formation of larval-pupal

intermediates or malformed pupae. All the pupae successfully metamorphosed to adults. However, following the emergence 21.54 percent adults (Table - 25) were malformed with underdeveloped as well as folded fore- and hind-wings. The right forewing was, however, less affected than others (Fig. 4, e). The total adult emergence was reduced by 8 percent as compared to control. Each of the affected females, on the average, laid 69.33 eggs less (Table - 26) than that of control. Fertility was reduced by 1.82 percent as compared to control. These changes in fecundity ($t = 1.49$ $p>0.05$) and fertility ($t = 1.58$, $p>0.05$) were statistically insignificant.

Further, 100 larvae were injected with 1.0 ug 'Triol' per larva. Then, 11 larvae died at various intervals of time and 8 larvae at larval-larval moult. The morphology and longevity of the larvae were unaffected. Again, 9 larvae of the 6th instar also died. At larval-pupal moult 6 more larvae died and 5 larval-pupal intermediates were formed (Fig. 3, a) which also could not survive. The rest of the larvae pupated normally and all the pupae metamorphosed to adults. The adult emergence was reduced by 12 percent as compared to the control. Among the adults, 14.75 percent (Table - 25) were malformed (Fig. 4, e). Each of the females emerged from treated larvae, on the average, laid 114.33 eggs less than that of control. But the fertility was 1.0 percent higher than that of control (Table - 26). The

observations on fecundity ($t = 2.27$, $p > 0.05$) and fertility ($t = 2.24$, $p > 0.05$) were statistically insignificant.

In the third group of 100 test larvae, each larva was injected with 2.0 ug 'Triol'. This dose caused the death of 13 larvae of the treated instar before next moulting and 10 larvae at moulting to 6th instar. Further, 8 larvae died at the 6th stage before pupation and 5 larvae at larval-pupal moult. However, the morphology and longevity of either instar remained unaffected. There were 6 larval-pupal intermediates formed at larval-pupal moult which also died. The remaining larvae pupated and 54 normal and 4 abnormal pupae (Table - 25) were formed. Later, 8 normal and all abnormal pupae failed to transform into adults. The malformed pupae had larval body shape and size. The adult emergence was reduced by 27 percent as compared to control and among the adults 34.8 percent (Table - 25) were malformed (Fig. 4, e). Each of the females (normally formed) emerged from the treated larvae, on average, laid 157.83 eggs less than that of control. Fertility was also reduced by 0.65 percent as compared to control (Table - 26). These observations on fecundity ($t = 2.16$, $p > 0.05$) and fertility ($t = 2.22$, $p > 0.05$) were statistically insignificant.

In the fourth group of 100 test larvae, each larva was injected with 4.0 ug 'Triol'. This dose caused mortality of 13 larvae of the treated instar at various intervals of time.

Further, 11 larvae died at next larval moult. The longevity and morphology of the treated larvae were unaffected. Then, 11 of the 6th instar larvae also died and 5 larvae further died at larval-pupal moult. There were 7 larval-pupal intermediates (Fig. 3, a) which could not survive longer and ultimately died. Following pupation, 47 normal and 6 abnormal pupae (Fig. 3, e) were formed. Later, 7 normal and all the abnormal pupae failed to metamorphose to adults. The adult emergence was reduced by 33 percent as compared to control. Also, 50 percent (Table-25) of the adults were malformed (Fig. 4, e). The fecundity of the affected females was significantly reduced by 288.0 eggs (average) per female ($t = 2.80$, $p<0.05$). Fertility was also significantly reduced by 29.23 percent (Table - 25) as compared to control ($t = 3.31$, $p<0.05$).

On injecting the strongest selected dose (6.0 ug 'Triol') to each of the 100 test larvae 15 larvae died during the treated instar. Further, 13 larvae died at next larval moult. But the morphology and longevity of the treated larvae were unaffected. Then 10 larvae died during the 6th instar. In this instar also the longevity and morphology of the larvae were unaffected. At larval-pupal moulting 8 larvae died and 10 larval-pupal intermediates (Fig. 3, a) were formed which could not pupate and died. Following pupation 40 pupae were normal and 4 pupae were abnormal. Out of these 4 normal and all

abnormal pupae (Fig. 3, e) failed to transform into adults. The adult emergence was reduced by 37 percent as compared to control. Also, 44.44 percent of the adults (Table - 25) were malformed (Fig. 4, e). The fecundity of the affected females was significantly reduced as each such female, on average, laid 327.83 eggs less than that of control ($t = 2.82$, $p<0.05$). Reduction in fertility was 40.77 percent (Table - 26) as compared to control which was statistically significant ($t = 3.31$, $p<0.05$).

(F) Effect of injection of different doses of 'Triol' on 6th instar larvae of Diacrisia obliqua:

Different doses of 'Triol' (0.5, 1.0, 2.0, 4.0 and 6.0 ug) were injected to each larva of 6th instar(24 hours old) D. obliqua. Similar larvae were injected with 1.0 ul acetone only to serve as parallel control. Each dose was tested against 100 larvae.

In the first group of 100 test larvae, 0.5 ug 'Triol' was injected to each larva. It was found that 18 larvae of the treated stage died following the injection of this dose whereas 18 larvae died at larval-pupal moulting. There was no change in the morphology and longevity of the larvae. The pupae were normal and all except 2 metamorphosed to adults. The adult emergence was reduced by 22 percent as compared to control. However, 3.22 percent adults (Table - 27) were malformed

(Fig. 4, f). Further, 6 affected females were paired separately with normal males (emerged from untreated larvae) of corresponding age for observation on fecundity. Then each of the affected females, on average, laid 37.50 eggs less than that of control. Reduction in fertility was 10.29 percent as compared to control (Table - 28). The fall in fecundity ($t = 0.95$, $p > 0.05$) and fertility ($t = 1.36$, $p > 0.05$) was statistically insignificant.

In the second group of 100 test larvae, each larva was injected with 1.0 ug 'Triol'. This dose caused the death of 13 larvae during the same stage and 12 larvae died at larval-pupal moult. The morphology and longevity of the larvae were not affected by this dose. Pupation was normal and all the pupae successfully metamorphosed to adults. The adult emergence was reduced by 9 percent as compared to control. Among the adults 6.66 percent (Table - 27) were malformed (Fig. 4, f). As regards the fecundity of the affected females each female, on average, laid 58.50 eggs less than that of control. Fertility was reduced by 7.91 percent as compared to control (Table - 28). The fall in fecundity ($t = 1.25$, $p > 0.05$) and fertility ($t = 1.80$, $p > 0.05$) was statistically insignificant.

In the third group of 100 test larvae, each larva was injected with 2.0 ug 'Triol'. The injection of this dose caused

the death of 19 larvae during this larval instar. Further, 10 larvae died at larval-pupal moult. The morphology and longevity of the larvae remained unaffected. Pupation was normal and all except 7 pupae transformed into adults. The adult emergence was reduced by 20 percent as compared to control. Among the adults 6.25 percent (Table - 27) were malformed (Fig. 4, f). Each of the affected females, on average, laid 89.83 eggs less than that of control. Fertility was, however, 1.67 percent higher than that of control. The data on fecundity ($t = 1.65$, $p > 0.05$) and fertility ($t = 1.60$, $p > 0.05$) were statistically insignificant.

Further, in the fourth group of 100 larvae 4.0 ug 'Triol' was injected to each larva. This dose caused the death of 18 larvae at different intervals of time. Later 17 larvae suffered mortality at next larval moulting. No variation either in morphology or longevity of the larvae was noticed. Further, 3 larval-pupal intermediates (Fig. 3, b) were also formed at pupation but these died. Pupation was normal but 10 pupae could not metamorphose to adults. The adult emergence was reduced by 32 percent as compared to control. Among the adults 11.54 percent (Table - 27) were malformed (Fig. 4, f). As regards fecundity and fertility of the females emerged from the treated larvae each of the affected females, on average, laid 161.66 eggs less than that of control whereas fertility

of the eggs was reduced by 31.55 percent as compared to control (Table - 28). The fall in fecundity ($t = 2.06$, $p>0.05$) was insignificant but that of fertility ($t = 3.27$, $p<0.05$) it was significant.

In the fifth group of 100 test larvae, each larva was injected with 6.0 ug 'Triol'. Then 22 larvae died during the larval duration whereas 22 larvae died at larval-pupal moult. The morphology and longevity of the larvae were not affected. Beside the formation of 5 larval-pupal intermediates (Fig.3,b) the pupation was otherwise normal. However, 10 pupae could not metamorphose to adults. The adult emergence was reduced by 43 percent as compared to control. Among the adults, 21.9 percent (Table - 27) were malformed (Fig. 4, f). Each of the affected females, on average, laid 389.0 eggs less than that of control which was statistically significant ($t = 2.97$, $p<0.05$). Fertility was reduced by 47.78 percent as compared to control (Table - 28). The reduction in fertility was statistically highly significant ($t = 3.72$, $p<0.05$).

(G) Effect of ingestion of different doses of 'Triol' on 5th instar larvae of Diacrisia obliqua:

From the stock culture of D. obliqua, newly moulted 5th instar larvae were removed to a fresh jar and then 24 hours old larvae were starved overnight. Such larvae were fed different doses of 'Triol' (0.5, 1.0, 2.0, 4.0 and 6.0 ug/larva)

as given in the section of "Materials and Methods". For control purpose larvae were fed on castor leaf treated with acetone only. Each dose was tested against 100 larvae.

Thus, in the first group of 100 test larvae, each larva was allowed to ingest 0.5 ug 'Triol'. Following the ingestion there was mortality of 9 larvae during the treated instar and 9 larvae at next larval moulting to 6th instar. The morphology and longevity of the surviving larvae were not affected. Further, 7 larvae died during the 6th instar and 5 larvae could not survive at larval-pupal moult. The morphology and longevity of the 6th instar larvae also remained unchanged. Pupation was normal. However, 8 pupae failed to metamorphose to adults and died. The rest of the pupae metamorphosed and emerged adults were normal. The adult emergence was, however, reduced by 9 percent as compared to control (Table - 29). Further, 6 affected females (emerged from treated larvae) were paired separately with normal males (emerged from untreated larvae) of corresponding age for observation on fecundity and fertility. It was found that each of the affected females, on average, laid 106.66 eggs less than that of control. Fertility was reduced by only 2.08 percent as compared to control (Table-30). The observation on fecundity ($t = 1.46$, $p>0.05$) and fertility ($t = 1.67$, $p>0.05$) was statistically insignificant.

In the second set of 100 test larvae, each larva was allowed to ingest 1.0 ug 'Triol'. Following this mortality was seen in 11 larvae of the treated instar at different intervals of time and in 8 larvae at moulting to the 6th instar. The morphology and longevity of the surviving 5th instar larvae were not affected by the ingestion of this dose. Then, 13 of the 6th instar larvae also died within 30 hours after moulting from 5th instar. Another 9 larvae of the 6th instar died at larval-pupal moult. The morphology and longevity of the surviving 6th instar larvae also remained unaffected. Pupation was normal and all pupae except 4 failed to metamorphose to adults. The adult emergence was reduced by 16 percent as compared to control. Among the adults 9.09 percent (Table - 29) were malformed with folded wings and extremely diminutive body (Fig. 4, g). Each of the affected females, on average, laid 95.66 eggs less than that of control. Fertility was also reduced by 2.47 percent as compared to control (Table - 30). These observations on fecundity ($t = 1.54$, $p>0.05$) and fertility ($t = 1.79$, $p>0.05$) were statistically insignificant.

In the third batch of test larvae, 2.0 ug 'Triol' was fed to each larva. Thereafter, 13 larvae died during the treated instar and 6 larvae perished at next larval moult. The morphology and longevity of the surviving treated larvae remained unchanged. Later 10 larvae of the 6th instar (moulted

from treated 5th instar) also died and another 8 larvae could not survive at larval-pupal moult. Pupation was normal and all except 8 pupae transformed into adults. The number of adults formed were 16 percent less as compared to control but of these 12.72 percent (Table - 29)adults were malformed (Fig.4,g). Further, each of the affected females, on average, laid 189.5 eggs less than that of control which is statistically insignificant ($t = 2.16$, $p > 0.05$). Fertility of the eggs was, however, 0.16 percent more than that of control (Table - 30) indicating insignificant change ($t = 2.23$, $p > 0.05$).

In the fourth set each of the 100 larvae was allowed to ingest 4.0 ug 'Triol'. Following the ingestion death of 15 larvae occurred at the same stage whereas 7 larvae died at next larval moult. The morphology of the larvae of treated instar was not affected but longevity was shortened by 24.32 hours. Later, 9 larvae of the 6th instar also died and finally 10 larvae died at larval-pupal moult. The remaining larvae pupated to 55 normal and 4 abnormal pupae. Then, 7 normal and 4 abnormal pupae (Fig. 3, H) could not metamorphose into adults. Finally, the number of adults after emergence was reduced by 19 percent as compared to control. However, 12.5 percent adults (Table - 29) were malformed (Fig. 4, g). Each of the affected females, on average, laid 134.5 eggs less than that of control (Table - 30). The fall in fecundity was insignificant ($t = 2.05$, $p > 0.05$). Fertility of the eggs was significantly reduced by 31.5 percent as compared to that of control

($t = 3.33$, $p<0.05$).

The ingestion of 6.0 ug 'Triol' by each of 100 larvae resulted in the death of 17 larvae during the same instar. Then, 6 larvae died at next larval moult. The morphology of the surviving larvae of the treated instar remained unaffected but their longevity was abbreviated by 36-40 hours as compared to control. Further, 10 larvae of the 6th instar died at various intervals of time and 7 larvae could not survive at larval-pupal moult. After the pupation 54 pupae were normal and 6 pupae were abnormal. Out of these 9 normal and 6 abnormal pupae (Fig. 2,H) failed to metamorphose to adults. The adults emerged were 26 percent less than that of control but 22.22 percent adults (Table - 29) were malformed (Fig.4,g). Each of the affected females, on average, laid 142.66 eggs less than that of control (Table - 30) which was insignificant fall ($t = 2.16$, $p>0.05$). Fertility of the eggs was reduced by 47.25 percent as compared to that of control which was statistically highly significant ($t = 3.91$, $p<0.05$).

(H) <u>Effect of ingestion of different doses of 'Triol' on 6th instar larvae of Diacrisia obliqua</u>:

As mentioned before similar doses of 'Triol' were fed to th 6th instar larvae of <u>D. obliqua</u> (24 hours old). The larvae ingesting leaf pieces treated with 1.0 ul acetone only served as parallel control. Each dose was tested against

100 larvae.

In the first group of 100 test larvae, each larva was fed with 0.5 ug 'Triol'. Following the ingestion of this dose death of 13 larvae occurred during this instar and that of 6 larvae at moulting to next instar. The morphology and longevity of the surviving larvae remained unchanged and their pupation was normal. There were 81 pupae out of which 78 pupae were normal and 3 pupae were abnormal. The abnormal pupae (Fig. 3,f) had pupal cuticle, telescoped segments, reduced and curved body. It was found that 5 normal pupae and all the abnormal pupae failed to metamorphose to adults. The adults emerged were 3 percent less as compared to control. Among the adults, 4.10 percent (Table - 31) were malformed (Fig. 4, h) with folded fore- and hind-wings. Further, 6 females emerged from the treated larvae were individually paired with equal number of normal males for observations on fecundity and fertility. Each affected female, on average, laid 71.83 eggs less than that of control (Table - 32) which was statistically insignificant ($t = 1.37$, $p > 0.05$). Fertility of the eggs was reduced by 4.79 percent as compared to control which was also insignificant change ($t = 1.60$, $p > 0.05$).

In the second group of 100 test larvae, each larva was fed with 1.0 ug,'Triol'. It was found that 11 larvae died during the treated instar whereas 11 larvae died at next moult.

There was no change in the morphology of the surviving larvae but the longevity of such larvae was shortened by 12-16 hours as compared to control. The remaining larvae pupated. Out of 78 pupae, 13 pupae were normal and 6 pupae were abnormal. The abnormal pupae (Fig. 3, g) retained larval abdominal appendages and a portion of larval cuticle. The size of these pupae was also reduced. It was found that 13 normal and all the 6 abnormal pupae did not metamorphose to adults. The adults emerged were 17 percent less as compared to that of control. Among the adults 13.55 percent were malformed (Fig. 4, h). Further, each of the affected females, on average, laid 111.5 eggs less than that of control (Table - 32). Fertility of the eggs was reduced by 0.40 percent as compared to control. The reduction in fecundity ($t = 1.86$, $p>0.05$) as well as fertility ($t = 1.80$, $p>0.05$) was statistically insignificant.

In the third group of 100 test larvae, each larva was fed with 2.0 ug 'Triol'. Following the ingestion of this dose there was mortality of 17 larvae at the treated instar and later 12 larvae at the next moult. The morphology of the surviving larvae remained unaffected but longevity of such larvae was shortened by 18-22 hours as compared to control. The remaining larvae pupated. Out of a total of 71 pupae, 65 pupae were normal and 6 pupae were malformed. Then 20 normal and all 6 malformed pupae (Fig. 3, g) failed to metamorphose to adults.

The number of adults was reduced by 31 percent as compared to control. Among the adults 13.33 percent were malformed (Fig. 4, h). Each of the affected females, on average, laid 239.33 eggs less than that of control (Table - 32) which was statistically highly significant ($t = 5.47$, $p<0.05$). Fertility of the eggs was also significantly reduced by 10.32 percent as compared to control ($t = 2.63$, $p<0.05$).

In the fourth group of 100 test larvae, each larva was fed with 4.0 ug 'Triol'. It was found that following the ingestion of this dose all the larvae developed lethargic conditions. Later, 6 larvae were reduced to very dimimutive forms (Fig. 3, e). All the dimimutive larvae and 10 other larvae died during the treated instar. Further, 8 larvae died at larval-pupal moult. The longevity of the surviving treated larvae was shortened by 22-26 hours as compared to control. This was followed by pupation and in all 76 pupae were formed. Out of these there were 64 normal and 12 abnormal pupae. Then 6 normal and 12 abnormal pupae (Fig. 3, g) could not metamorphose to adults. The number of adults was reduced by 18 percent as compared to control (Table - 31) in which 17.24 percent were malformed (Fig. 4, h). Each of the affected females, on average laid 309.33 eggs less than that of control (Table - 32) which was statistically significant ($t = 3.05$, $p<0.05$). Fertility was also significantly reduced by 6.07 percent as compared to control ($t = 2.83$, $p<0.05$).

In the fifth group of 100 test larvae, 6.0 ug 'Triol' was fed to each larva. Following the ingestion all the larvae developed lethargic condition. The size of 10 larvae was extremely reduced and consequently they died. Then another 11 larvae died later. At larval-pupal moult 11 larvae of the treated instar further died. The longevity of the treated instar was abbreviated by 30-35 hours as compared to control. The remaining larvae pupated. Thus in all 68 pupae were formed. Among these 53 pupae were normal and 15 pupae were abnormal. Out of these 15 normal pupae metamrophosed partially resulting in the formation of pupal-adult intermediates (Fig. 3, i and Fig. 3, j). None of the malformed pupae (Fig. 3, h) could metamorphose to adults. The adults were 38 percent less in number as compared to control (Table - 31). Among the adults 21.05 percent were malformed (Fig. 4, h & Fig. 3, k). Each of the affected females, on average, laid 364.0 eggs less than that of control (Table - 32). Fertility of the eggs was also reduced by 12.51 percent as compared to control. The fall in fecundity ($t = 3.91$, $p < 0.05$) as well as fertility ($t = 4.24$, $p < 0.05$) was highly significant.

Table - 17: Showing larval mortality, larval-pupal intermediates, pupal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of α-ecdysone to the 5th instar larvae of Diacrisia obliqua (Each dose was tested against 100 larvae).

Dose/larva (µg)	Larval Mortality (Number)					Pupation (Number)		Pupal Mortality (Number)		Adult emergence (Number)		Total loss up to emergence (%)
	5th instar	At next moult	6th instar	At larval-pupal moult		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
				Normal larvae	Larval-pupal intermediates							
Control	13	-	6	-	-	81	-	4	-	77	-	23
0.5	10	5	9	3	-	73	-	4	-	69	-	31
1.0	9	6	10	2	4	65	4	4	3	62	-	38
2.0	10	5	9	3	7	63	3	3	3	52	8	40
4.0	12	5	8	4	5	61	5	5	5	50	6	44
6.0	13	5	6	6	12	48	10	7	10	30	11	59

Average of 5 replicates

Table - 18: Showing fecundity and fertility of females of *Diacrisia obliqua* emerged from 5th instar larvae injected with different doses of α-ecdysone.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	784.00 ± 20.11	773.33 ± 16.14	98.63
0.5	772.33 ± 18.33	726.83 ± 29.84	94.10
1.0	719.16 ± 36.51	698.16 ± 39.43	97.07
2.0	674.50 ± 55.91	633.16 ± 52.81	93.87
4.0	602.16 ± 61.38	557.50* ± 57.91	92.58
6.0	625.33 ± 73.92	565.16 ± 64.02	90.37

* Significant at 5% level

Table - 19: Showing larval mortality, larval-pupal intermediates, pupal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of α-ecdysone to the 6th instar larvae of Diacrisia obliqua (Each dose was tested against 100 larvae).

Dose/larva (µg)	Larval Mortality (Number)			Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss up to emergence (%)
	6th instar	At larval-pupal moult		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
		Normal larvae	Larval-pupal intermediates							
Control	7	6	-	87	-	3	-	84	-	16
0.5	11	5	-	84	-	7	-	77	-	23
1.0	9	5	5	81	-	9	-	66	6	28
2.0	8	8	4	80	-	8	-	72	-	28
4.0	12	7	8	73	-	8	-	60	5	35
6.0	15	6	6	73	-	9	-	55	9	36

Average of 5 replicates

Table - 20: Showing fecundity and fertility of females of *Diacrisia obliqua* emerged from 6th instar larvae injected with different doses of α-ecdysone.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	779.83 ± 22.26	750.83 ± 19.98	96.28
0.5	714.33 ± 45.42	680.33 ± 45.02	95.24
1.0	713.66 ± 32.05	661.16 ± 26.42	92.64
2.0	692.66 ±17.62	667.66 ± 20.71	96.39
4.0	608.66* ± 22.69	582.00* ± 29.05	95.61
6.0	510.16 ±125.63	447.33 ±111.04	87.68

* Significant at 5% level

Table - 21: Showing larval mortality, larval-pupal intermediates, pupal mortality, adult emergence and total loss up to adult emergence following the ingestion of different doses of α-ecdysone by the 5th instar larvae of Diacrisia obliqua (Each dose was tested against 100 larvae).

Dose/larva (ug)	Larval Mortality (Number)					Pupation (Number)		Pupal Mortality (Number)		Adult emergence (Number)		Total loss up to emergence (%)
	5th instar	At next moult	6th instar	At larval-pupal moult								
				Normal larvae	Larval-pupal intermediates	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	10	2	6	-	-	82	-	-	-	82	-	18
0.5	10	4	4	15	-	67	-	4	-	63	-	37
1.0	15	5	6	12	-	62	-	8	-	54	-	46
2.0	17	6	8	16	-	53	-	12	-	41	-	59
4.0	20	5	10	19	-	46	-	9	-	37	-	63
6.0	22	8	7	23	-	40	-	11	-	20	9	71

Average of 5 replicates

Table - 22: Showing fecundity and fertility of females of *Diacrisia obliqua* emerged from 5th instar larvae ingesting different doses of α-ecdysone.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	803.00 ± 39.97	784.00 ± 40.26	97.63
0.5	757.66 ± 36.91	723.16 ± 37.24	95.44
1.0	645.66 ± 35.88	626.83 ± 38.23	97.08
2.0	669.33 ± 17.32	632.83 ± 16.86	95.38
4.0	577.66* ± 19.01	556.33* ± 16.69	96.30
6.0	582.83* ± 17.43	555.83* ± 18.51	95.36

* Significant at 5% level

Table - 23: Showing larval mortality, larval-pupal intermediates, pupal mortality, adult emergence and total loss up to adult emergence following the ingestion of different doses of α-ecdysone by the 6th instar larvae of Pieris obliqua (Each dose was tested against 100 larvae).

Dose/larva (ug)	Larval Mortality (Number)			Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss up to emergence (%)
	6th instar	At larval-pupal moult		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
		Normal larvae	Larval-pupal intermediates							
Control	18	-	-	82	-	3	-	79	-	21
0.5	20	4	-	76	-	1	-	75	-	25
1.0	21	-	-	79	-	8	-	71	-	29
2.0	22	3	-	75	-	11	-	64	-	36
4.0	30	6	-	64	-	8	-	56	-	44
6.0	32	6	-	62	-	11	-	38	13	49

Average of 5 replicates

Table - 24: Showing fecundity and fertility of females of Diacrisia obliqua emerged from 6th instar larvae ingesting different doses of α-ecdysone.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	868.83 ± 19.55	847.83 ± 16.52	97.58
0.5	782.00 ± 33.39	759.33 ± 26.48	97.10
1.0	797.33 ± 14.84	735.33* ± 15.77	92.22
2.0	740.33* ± 18.69	711.83* ± 11.68	96.15
4.0	863.50* ± 33.58	836.50* ± 20.65	96.94
6.0	833.66* ± 33.12	803.16* ±26.92	94.26

* Significant at 5% level.

Table - 25: Showing larval mortality, larval-pupal intermediates, pupal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of 'Triol' to the 5th instar larvae of Diacrisia obliqua (Each dose was tested against 100 larvae).

Dose/larva (ug)	Larval Mortality (Number)					Pupation (Number)		Pupal Mortality (Number)		Adult emergence (Number)		Total loss up to emergence (%)
	5th instar	At next moult	6th instar	At larval-pupal moult								
				Normal larvae	Larval-pupal intermediates	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	5	2	11	9	-	73	-	-	-	73	-	27
0.5	9	9	6	11	-	65	-	-	-	51	14	35
1.0	11	8	9	6	5	61	-	-	-	52	9	39
2.0	13	10	8	5	6	54	4	8	4	30	16	54
4.0	13	11	11	5	7	47	6	7	6	20	20	60
6.0	15	13	10	8	10	40	4	4	4	20	16	64

Average of 5 replicates

Table - 26: Showing fecundity and fertility of females of *Diacrisia obliqua* emerged from 5th instar larvae injected with different doses of 'Triol'.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	504.33 ± 43.04	492.83 ± 42.26	97.71
0.5	435.00 ± 33.41	417.16 ± 31.92	95.89
1.0	390.00 ± 11.24	385.00 ± 10.02	98.71
2.0	346.50 ± 39.25	336.33 ± 36.91	97.06
4.0	216.33* ± 46.95	148.16* ± 36.00	68.48
8.0	176.50* ± 57.56	100.50* ± 44.06	56.94

* Significant at 5% level

Table - 27: Showing larval mortality, larval-pupal intermediates, pupal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of 'Triol' to the 6th instar larvae of Diacrisia obliqua (Each dose was tested against 100 larvae).

Dose/ larva (ug)	Larval Mortality (Number)			Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss up to emergence (%)
	6th instar	At larval-pupal moult		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
		Normal larvae	Larval-pupal intermediates							
Control	9	7	-	84	-	-	-	84	-	16
0.5	18	18	-	64	-	2	-	62	-	38
1.0	13	12	-	75	-	-	-	70	5	25
2.0	19	10	-	71	-	7	-	60	4	36
4.0	18	17	3	62	-	10	-	46	6	48
6.0	22	22	5	51	-	10	-	32	9	59

Average of 5 replicates

Table - 28: Showing fecundity and fertility of females of *Diacrisia obliqua* emerged from 6th instar larvae injected with different doses of 'Triol'.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	512.66 ± 50.24	497.83 ± 48.94	97.10
0.5	475.16 ± 48.59	412.50 ± 62.31	86.81
1.0	454.16 ± 40.90	406.16 ± 20.87	89.21
2.0	422.83 ± 30.34	417.66 ± 27.59	98.77
4.0	351.00 ± 42.77	195.00* ± 20.13	55.55
6.0	123.66* ± 57.25	61.00* ± 28.12	49.32

* Significant at 5% level

Table - 29: Showing larval mortality, larval-pupal intermediates, pupal mortality, adult emergence and total loss up to adult emergence following the ingestion of different doses of 'Triol' by the 5th instar larvae of Diacrisia obliqua (Each dose was tested against 100 larvae).

Dose/larva (ug)	Larval Mortality (Number)					Pupation (Number)		Pupal Mortality (Number)		Adult emergence (Number)		Total loss up to emergence (%)
	5th instar	At next moult	6th instar	At larval-pupal moult		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
				Normal	Larval-pupal intermediate							
Control	7	5	9	8	-	71	-	-	-	71	-	29
0.5	9	9	7	5	-	70	-	8	-	62	-	38
1.0	11	8	13	9	-	59	-	4	-	50	5	45
2.0	13	6	10	8	-	63	-	8	-	48	7	45
4.0	15	7	9	10	-	55	4	7	4	42	6	52
6.0	17	6	10	7	-	54	6	9	6	35	10	55

Average of 5 replicates

Table - 30: Showing fecundity and fertility of females of Diacrisia obliqua emerged from 5th instar larvae ingesting different doses of 'Triol'.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	685.16 ± 51.39	664.50 ± 39.58	96.98
0.5	578.50 ± 70.32	549.00 ± 61.10	94.90
1.0	589.50 ± 45.93	557.16 ± 41.92	94.51
2.0	495.66 ± 47.63	481.50 ± 50.30	97.14
4.0	490.66 ± 61.57	321.33* ± 36.15	65.48
6.0	536.50 ± 72.85	266.83* ± 23.90	49.73

* Significant at 5% level

Table - 31: Showing larval mortality, larval-pupal intermediates, pupal mortality, adult emergence and total loss up to adult emergence following the ingestion of different doses of 'Triol' by the 6th instar larvae of Diacrisia obliqua (Each dose was tested against 100 larvae).

Dose/ larva (ug)	Larval Mortality (Number)			Pupation (Number)		Pupal Mortality (Number)		Adult Emergence (Number)		Total loss up to emergence (%)
	6th instar	At larval-pupal moult		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
		Normal larvae	Larval-pupal intermediate							
Control	7	7	-	86	-	10	-	76	-	24
0.5	13	6	-	78	3	5	3	70	3	27
1.0	11	11	-	72	6	13	6	51	8	41
2.0	17	12	-	65	6	20	6	39	6	55
4.0	16	8	-	64	12	6	12	48	10	42
6.0	21	11	-	53	15	15	15	30	8	62

Average of 5 replicates

Table - 30a Showing fecundity and fertility of females of Diagrisia obliqua emerged from 6th instar larvae ingesting different doses of 'Triol'.

Dose/larva (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	744.16 ± 42.69	719.50 ± 46.39	96.68
0.8	672.33 ± 49.77	617.83 ± 50.65	91.89
1.0	632.66 ± 35.90	609.16 ± 38.94	[illegible]
2.0	504.83* ± 64.37	436.00* ± 53.52	[illegible]
4.0	434.83* ± 38.49	394.00* ± 32.82	90.61
6.0	380.16* ± 15.54	320.00* ± 7.87	84.17

*Significant at 5% level

Fig. 2 Larval and pupal malformation in Diacrisia oblicua following the treatment with -ecdysone and 'Triol'.

Fig. 3 Larval-pupal intermediates, malformed pupae and pupal-adult intermediates in Diacrisia oblicua following the treatment with -ecdysone and 'Triol'.

Fig. 4 Malformation in the adults of Diacrisia oblicua.

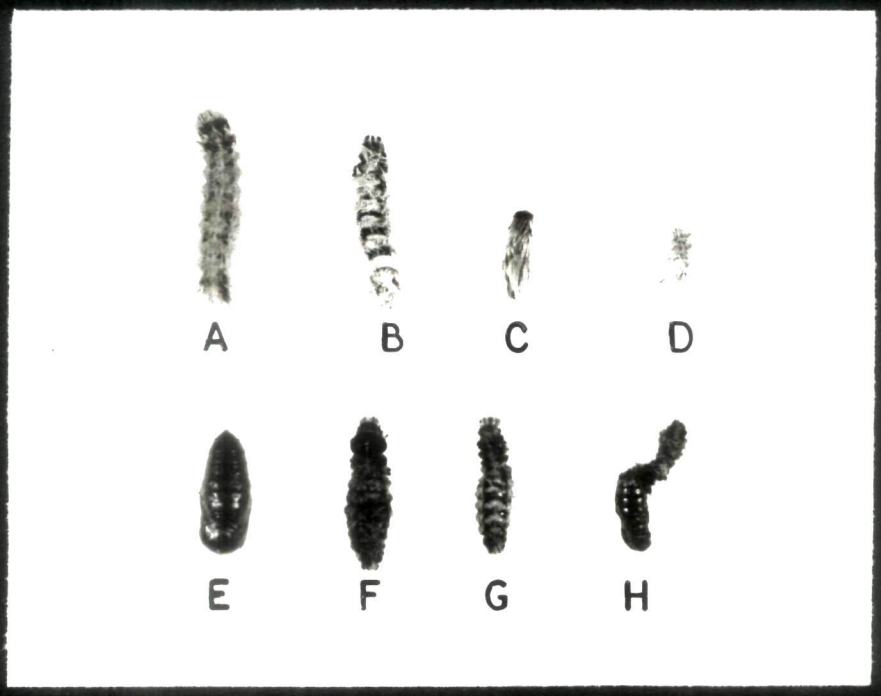

FIG. 2

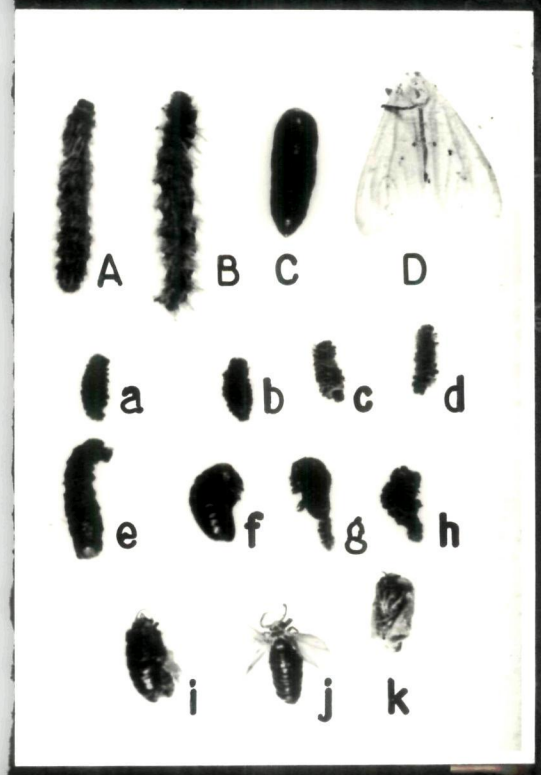

FIG. 3

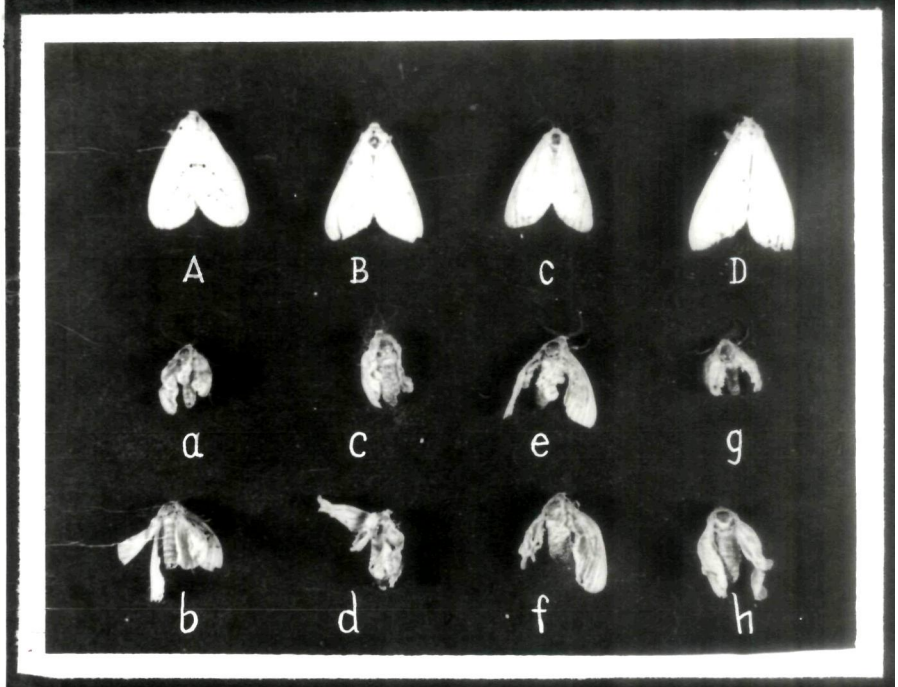

FIG. 4

3. Dysdercus cingulatus:

(A) Effect of injection of different doses of ∝-ecdysone on 4th instar nymphs of Dysdercus cingulatus:

Similar to the experiments on Spodoptera litura and Diacrisia obliqua, nymphal stages (4th and 5th instars) of D. cingulatus were also injected with selected doses of ∝-ecdysone (0.5, 1.0, 2.0, 4.0 and 6.0 ug/nymph), which were the same as used in S. litura and D. obliqua. Each dose was applied on 100 nymphs of 4th instar (24 hours old). Acetone treated nymphs served as parallel control.

In the first test set, 100 nymphs of 4th instar were injected with 0.5 ug ∝-ecdysone/nymph. It was found that 3 nymphs died during the same stage whereas 1 nymph died at the subsequent nymphal moult. The morphology and longevity of the treated 4th instar nymphs were not affected. When the nymphs entered 5th instar, again 2 nymphs died whereas mortality of 2 nymphs occurred at nymphal-adult moult. The morphology and longevity of 5th instar nymphs were also normal. There was a loss of 3 percent in adult number obtained from the treated nymphs as compared to that of control (Table - 33). For recording the fecundity and fertility, the procedure was the same as described before in case of S. litura and D. obliqua. Thus 6 females emerged from the treated nymphal stock were paired with similar number of males from untreated stock. On the average each

affected female laid 8.5 eggs less than that of control (Table - 34) which was a insignificant fall ($t = 1.08$, $p > 0.05$). Fertility of the eggs was reduced by 3.17 percent as compared to control which was also a insignificant change ($t = 1.46$, $p > 0.05$).

In the second batch of test nymphs, each nymph was injected with 1.0 ug α-ecdysone. Then, 5 nymphs died at next nymphal moult. Further, 3 nymphs suffered mortality at next nymphal instar and 1 nymph died at final moulting to adult. No change either in morphology or longevity of the nymphal instar was noticed. The number of adults was 6 percent less as compared to that of control (Table - 33). Each of the affected females, on average, laid 3.17 eggs less than that of control (Table - 34). Fertility of the eggs was reduced by 3.56 percent as compared to control. The fall in fecundity ($t = 0.73$, $p > 0.05$) and fertility ($t = 1.10$, $p > 0.05$) was statistically insignificant.

In the third set of insects, each of the 100 nymphs was injected with 2.0 ug α-ecdysone. It was found that 7 nymphs died during the same stage whereas 2 nymphs died at next nymphal moult. Again 2 nymphs died during the 5th instar and finally 2 more nymphs died at nymphal-adult moulting. The morphology and longevity of the nymphs during both nymphal instars remained unaffected. The emergence of adults was,

however, reduced by 8 percent as compared to control (Table-33). Further, each of the affected females, on average, laid 7.17 eggs less than that of control. Fertility of the eggs was reduced by 6.24 percent (Table - 34) as compared to control. The fall in fecundity ($t = 0.95$, $p>0.05$) and fertility ($t = 1.55$ $p>0.05$) was statistically insignificant.

In the fourth group of test insects, each of the 100 nymphs was injected with 4.0 ug -ecdysone. Then, 9 nymphs died at the treated stage whereas 5 nymphs died at next nymphal moult. Further, 5 nymphs could not survive during the 5th instar and 2 nymphs died at nymphal-adult moult. However, there was no change either in longevity or morphology of the nymphs during either instar. The adult number was 16 percent less than that of control (Table - 33). It was also found that each of the affected females, on average, laid 4.34 eggs less than that of control indicating a insignificant fall ($t = 0.80$, $p>0.05$). Similarly, fertility of the eggs was also reduced by 3.2 percent as compared to control (Table - 34), which was also a insignificant change ($t = 1.16$, $p>0.05$).

In the fifth set of test insects, each of the 100 nymphs was injected with 6.0 ug α-ecdysone. This resulted in the mortality of 15 nymphs at the same stage and 3 nymphs at next nymphal moult. Then, 4 nymphs again suffered mortality at 5th nymphal instar and finally 5 nymphs died at moulting to adult.

The morphology and longevity of the nymphs were not affected. However, the adult formation was reduced by 22 percent (Table-33) as compared to control. Each of the affected females, on average, laid 12.34 eggs less than that of control (Table - 34) showing insignificant fall ($t = 1.25$, $p > 0.05$). Fertility of the eggs was also reduced by 7 percent as compared to control which was also a insignificant fall ($t = 1.91$, $p > 0.05$).

(B) Effect of injection of different doses of ∝-ecdysone on 5th instar nymphs of Dysdercus cingulatus:

As mentioned in the preceding section 5th instar nymphs of D. cingulatus were also injected with similar doses of ∝-ecdysone. For parallel control nymphs of similar stage were injected with acetone only.

In the first batch of test insects, each of the 100 nymphs was injected with 0.5 ug ∝-ecdysone. Following this 6 nymphs died during the same instar and 5 nymphs could not survive at nymphal-adult moulting. There was no change in the morphology and longevity of the treated nymphs of 5th instar. However, adult emergence was 5 percent less as compared to control (Table - 35). Each of the affected females (emerged from the treated nymphs), on average, laid 3.34 eggs less than that of control (Table - 36) showing insignificant fall ($t = 0.81$, $p > 0.05$). Fertility of the eggs was reduced by 0.10 percent which was also a insignificant change ($t = 0.88$, $p > 0.05$).

In the second group of test insects, each nymph was injected with 1.0 ug α-ecdysone. It was found that 6 nymphs died during the treated instar whereas 4 nymphs further died at nymphal-adult moult. The morphology and longevity of the surviving nymphs remained unaffected. The number of adults emerged from the treated nymphs was 3 percent less as compared to control (Table - 35). Further, each of the affected females, on average, laid 17.0 eggs less than that of control (Table-36). Fertility of the eggs was also reduced by 6.01 percent as compared to control. The fall in both fecundity ($t = 1.96$, $p>0.05$) and fertility ($t = 2.43$, $p>0.05$) was statistically insignificant.

In the third test set, each of the 100 nymphs was injected with 2.0 ug α-ecdysone. Then 12 nymphs suffered mortality during the same instar and another 11 nymphs died at nymphal-adult moult. The morphology and longevity of the surviving treated nymphs remained unaffected. The number of adults emerged from the treated nymphs was 17 percent less as compared to control (Table - 35). Each of the affected females, on average, laid 23.0 eggs less than that of control (Table-36). Fertility of the eggs was reduced by 5.62 percent as compared to control. The change in fecundity ($t = 2.11$, $p>0.05$) was statistically insignificant but that of fertility ($t = 2.68$, $p<0.05$) was significant.

In the fourth set, each nymph was injected with 4.0 ug ∝-ecdysone. It was found that 11 nymphs died during treated 5th instar whereas 10 nymphs died later at nymphal-adult moulting. There was no change in the morphology and longevity of the surviving nymphs. The fecundity of the females, on average, dropped by 31.84 eggs as compared to control (Table.36), which was a insignificant loss ($t = 2.07$, $p>0.05$). Fertility of the eggs was reduced by 14.50 percent as compared to control which was statistically significant ($t = 2.72$, $p<0.05$).

In the fifth set, each nymph was injected with 6.0 ug ∝-ecdysone. Thereafter 28 nymphs died during the same instar whereas 9 nymphs could not survive at nymphal-adult moulting. The morphology and longevity of the surviving nymphs were unaffected. However, the adult emergence was reduced by 31 percent as compared to control (Table - 35). Each of the affected females, on average, oviposited 49.84 eggs less than that of control. Fertility of the eggs was also reduced by 21.74 percent as compared to control (Table - 36). The changes both in fecundity ($t = 2.59$, $p<0.05$) and fertility ($t = 3.87$, $p<0.05$) were statistically significant.

(C) Effect of topical application of different doses of ∝-ecdysone on 4th instar nymphs of Dysdercus cingulatus:

To observe the effect of ∝-ecdysone on moulting, metamorphosis, fecundity and fertility of D. cingulatus,

different doses of α-ecdysone were topically applied on the 4th as well as 5th instar nymphs. The different doses were the same (0.5, 1.0, 2.0, 4.0 and 6.0 ug/nymph) as used in case of S. litura and D. obliqua larvae. In the present section, observations following the treatment of newly moulted 4th instar nymphs have been described below. Control insects were treated with acetone only. Each dose was tested against 100 nymphs.

In the first group of 100 nymphs each nymph was treated with 0.5 ug α-ecdysone. This dose caused the mortality of 6 nymphs during the same instar and 4 nymphs at next nymphal moult. The morphology and longevity of the treated nymphs which survived were not affected. Then 3 nymphs of the 5th instar (moulted from 4th instar) also died and finally 2 nymphs died at moulting to adult stage. The morphology and longevity of 5th instar nymphs also remained unchanged. The adult emergence was 5 percent less as compared to control (Table - 37). For estimating fecundity and fertility, 6 females emerged from the treated nymphs were paired with males of similar age which emerged from untreated nymphs. It was found that each affected female, on average, laid 5.33 eggs less (Table - 38) than that of control which was statistically insignificant ($t \neq 0.61$, $p > 0.06$). Fertility was, however, 3.51 percent higher than that of control which was also statistically insignificant

($t = 0.11$, $p > 0.05$).

In the second batch of 100 test nymphs, each nymph was treated with 1.0 ug α-ecdysone. Then 9 nymphs died during this instar and 5 nymphs at next nymphal moult. Further, 6 nymphs suffered mortality during the next instar and 5 nymphs died at final moulting to adults. The morphology and longevity of both the nymphal instars were unaffected. The adult emergence from the treated nymphs was 15 percent less as compared to control (Table - 37). The reduction in fecundity of the affected females was insignificant as each of such female, on average, laid 6.0 eggs less than that of control ($t = 0.69$, $p > 0.05$). Fertility of the eggs was 1.75 percent higher as compared to that of control (Table - 38) which was statistically insignificant ($t = 0.50$, $p > 0.05$).

In the third batch of test nymphs, each nymph was treated with 2.0 ug α-ecdysone. This dose caused the death of 12 nymphs during the treated instar and 4 nymphs at the nymphal moulting to 5th instar. Again, 5 nymphs died during the 5th instar and 6 nymphs at nymphal-adult moulting could not survive. However, there was no change in either morphology or longevity of the surviving nymphs of either instar. The adults emerged were 17 percent less than that of control (Table - 37). The reduction in fecundity of the affected females was insignificant as each such female, on average, laid 6.5 eggs less than

that of control ($t = 0.88$, $p > 0.05$). Fertility of the eggs was insignificantly reduced by 7.28 percent as compared to control ($t = 1.33$, $p > 0.05$).

In the fourth group of 100 nymphs, each nymph was treated with 4.0 ug α-ecdysone. Following this treatment 15 nymphs died during the treated instar and 4 nymphs at next nymphal moult. Further, 8 nymphs of the 5th instar also died and finally 4 nymphs could not survive at nymphal-adult moulting. No change in morphology and longevity was noticed in the surviving nymphs of either instar. When the adults emerged out of the treated nymphs these were 21 percent less than that of control (Table - 37). Each of the affected females, on average, laid 13.5 eggs less than that of control. Fertility of the eggs was reduced by 4.18 percent as compared to control (Table - 38). The observations on fecundity ($t = 1.01$, $p > 0.05$) and fertility ($t = 1.16$, $p > 0.05$) were statistically insignificant.

In the fifth group, each of the 100 nymphs was treated with 6.0 ug α-ecdysone. It was found that 20 nymphs died during the treated instar and 6 nymphs more perished at moulting to 5th instar. Later, 10 nymphs died during the 5th instar and 5 nymphs suffered mortality at moulting to adult stage. There was no change in the morphology and longevity of the nymphs of either instar. The adults emerged were 31 percent less as

compared to control (Table - 37). The reduction in fecundity was insignificant as each of such female, on average, laid 18.0 eggs less than that of control ($t = 1.19$, $p > 0.05$). Fertility of the eggs was also insignificantly reduced by 10.24 percent (Table - 38) as compared to control ($t = 1.67$, $p > 0.05$).

(D) Effect of topical application of different doses of -ecdysone on 5th instar nymphs of Dysdercus cingulatus:

Newly moulted 5th instar nymphs of D. cingulatus were treated with selected doses of ∝-ecdysone. Each dose was applied on 100 nymphs. Control insects were treated with acetone only as described in the section of "Materials and Methods".

In the first group of 100 test insects, each insect was topically treated with 0.5 ug ∝-ecdysone. It was found that 8 nymphs died during the same instar and 3 nymphs could not survive at nymphal-adult moult. The morphology and longevity of the treated nymphs remained unchanged. The number of adults emerged out of the treated nymphs were 6 percent less as compared to that of control (Table - 39). The fecundity of the affected females was insignificantly reduced as each such female, on average, laid 8.0 eggs less than that of control ($t = 0.92$, $p > 0.05$). The fertility of the eggs was also insignificantly reduced by 8.34 percent (Table - 40) as compared to

control ($t = 1.51$, $p > 0.05$).

In the second group of 100 test insects, each nymph was treated with 1.0 ug α-ecdysone. Following the treatment there was mortality of 14 nymphs during the same instar and 5 nymphs at nymphal-adult moult. The morphology and longevity of the treated nymphs which survived were not affected. Finally the number of adults emerged was 14 percent less than that of control (Table - 39). Each of the affected females, on average, laid 15.0 eggs less than that of control (Table - 40) which was insignificant change ($t = 1.22$, $p > 0.05$). Fertility of the eggs was also insignificantly reduced by 16.01 percent as compared to control ($t = 1.88$, $p > 0.05$).

In the third group, each of 100 nymphs was treated with 2.0 ug α-ecdysone. Out of these 23 nymphs died during the same instar and 4 nymphs at nymphal-adult moult. Though the morphology of the surviving nymphs remained unchanged, the longevity of the treated nymphs was shortened by 12-15 hours as compared to control. The adults emerged were 22 percent less than that of control (Table - 39). Fecundity of the affected females was insignificantly reduced as each such female, on average, laid 18.83 eggs less (Table - 40) than that of control ($t = 1.46$, $p > 0.05$). Fertility of the eggs was reduced by 17.93 percent as compared to control ($t = 2.33$ $p > 0.05$) which statistically insignificant.

In the fourth group of 100 test nymphs, each nymph was treated with 4.0 ug α-ecdysone. It was found that 27 nymphs died during the same instar and 4 nymphs perished at moulting to adult. The morphology of the surviving nymphs remained unaffected but longevity was shortened by 18-24 hours as compared to control. Fecundity of the affected females was insignificantly reduced as each such female, on average, laid 32.83 eggs less (Table - 39) than that of control ($t = 2.19$, $p > 0.05$). There was 28.04 percent reduction in fertility which was statistically significant ($t = 2.85$, $p < 0.05$).

In the fifth group, each of the 100 nymphs was treated with 6.0 ug α-ecdysone. Following the treatment 30 nymphs during the same instar and 6 nymphs at nymphal-adult moult died. The morphology of the treated instar was not affected but longevity was shortened by 25-30 hours as compared to control. The adult emergence was reduced by 31 percent as compared to control (Table - 39). Each of the affected females, on average, laid 68.33 eggs less than that of control (Table-40) which was statistically insignificant ($t = 2.14$, $p > 0.05$). Fertility of the eggs was reduced by 31.18 percent as compared to control which was statistically significant ($t = 2.66$, $p < 0.05$).

(E) Effect of injection of different doses of 'Triol' on 4th instar nymphs of Dysdercus cingulatus:

Selected doses of 'Triol' (0.5, 1.0, 2.0, 4.0 and 6.0 ug), an analogue of moulting hormone, were injected to individual nymphs of 4th instar D. cingulatus. The doses were similar to those used for S. litura and D. obliqua. Each dose was tested against 100 nymphs, 24 hours old. Control insects were injected with acetone only as described in the section of "Materials and Methods".

Thus, in the first test set, each nymph was injected with 0.5 ug 'Triol'. It was found that 11 nymphs died during the treated instar and 7 nymphs died at next nymphal moulting. Again, 9 nymphs died at next nymphal instar whereas mortality of 6 nymphs occurred at nymphal-adult moult. There was no change in the morphology and longevity of either instar. The formation of adults was 18 percent less than that of control (Table - 41). Each of the affected females (emerged from the treated nymphal stock), on average, laid 1.5 eggs less than that of control (Table - 42) which was a insignificant fall ($t = 0.45$, $p>0.05$). Fertility of the eggs was reduced by 2.24 percent as compared to control which was also a insignificant loss ($t = 0.77$, $p>0.05$).

In the second test set, each nymph was injected with 1.0 ug 'Triol'. The injection caused the death of 14 nymphs during the treated stage and 8 nymphs at next nymphal moulting.

Again, 9 nymphs died at next nymphal instar whereas 11 nymphs could not survive at final moulting to adult. The morphology and longevity of the nymphs at both the instars remained unaffected. The adults formed were 27 percent less as compared to control (Table - 41). Each of the affected females, on average, laid 6.50 eggs less than that of control (Table - 42). Fertility of the eggs was reduced by 11.22 percent as compared to control. The data on fecundity ($t = 0.97$, $p > 0.05$) and fertility ($t = 1.80$, $p > 0.05$) were statistically insignificant.

In the third test set, each nymph was injected with 2.0 ug 'Triol'. Following the injection 19 nymphs suffered mortality at the same stage and 7 nymphs at next nymphal moult. Thereafter 13 nymphs during next instar and 16 nymphs at nymphal moulting to adult also died. The morphology and longevity of either instar were not affected. There was 40 percent reduction in the emerged adults as compared to control (Table - 41). Each of the affected females, on average, laid 2.0 eggs less than that of control (Table - 42) which was a insignificant fall ($t = 0.57$, $p > 0.05$). Fertility of the eggs was also insignificantly reduced by 17.33 percent as compared to control ($t = 2.02$, $p > 0.05$).

In the fourth batch of test insects, each nymph was injected with 4.0 ug 'Triol'. It was found that 23 nymphs of the treated instar died at different intervals of time. Further,

9 nymphs died at next nymphal moulting. At the next instar also, 17 nymphs died. Finally 16 nymphs suffered mortality at nymphal-adult moulting. The morphology and longevity of the nymphal instars were not changed. The number of adults obtained from the treated nymphs was 30 percent less as compared to that of control (Table - 41). Each of the affected females, on average, laid 20.83 eggs less than that of control (Table - 42) which was a insignificant loss ($t = 1.62$, $p > 0.05$). Fertility of the egg was, however, significantly reduced by 29.63 percent as compared to control ($t = 3.03$, $p < 0.05$).

In the fifth group of test insects, each nymph was injected with 6.0 ug 'Triol'. Following this injection 39 nymphs died during the treated instar. At the next nymphal moult 13 nymphs suffered mortality. Further, 18 nymphs died during the 5th instar and 19 nymphs suffered mortality at nymphal-adult moult. However, there was no change in morphology and longevity of the nymphs of either 4th or 5th instar. There was 64 percent reduction in the number of adults (Table - 41). Each of the affected females which emerged from the treated stock of nymphs, on average, laid 24 eggs less than that of control (Table - 42) which was insignificant loss ($t = 1.84$, $p > 0.05$). Fertility of the eggs was significantly reduced by 39.92 percent as compared to control ($t = 3.17$, $p < 0.05$).

(F) Effect of injection of different doses of 'Triol' on 5th instar nymphs of Dysdercus cingulatus:

Different doses of 'Triol' (0.5, 1.0, 2.0, 4.0 and 6.0 ug/nymph) were injected to the 5th instar nymphs of Dysdercus cingulatus. Each dose was tested against 100 nymphs (24 hours old). Control insects were injected with acetone only.

In the first group of 100 nymphs, 0.5 ug 'Triol' was injected to each nymph. It was found that 11 nymphs died during the same instar and 8 nymphs could not survive at nymphal-adult moulting. No effect on morphology or longevity of the nymphs was noticed. The number of adults which emerged from the treated nymphs was 11 percent less as compared to control (Table - 43). Each of the affected females, on averge, laid 13.16 eggs less as compared to control (Table - 44) which was a insignificant loss ($t = 1.11$, $p>0.05$). Fertility of the eggs was insignificantly reduced by 7.07 percent as compared to control ($t = 1.62$, $p>0.05$).

In the second group of 100 nymphs, each nymph was injected with 1.0 ug 'Triol'. Following the injection of this dose there was death of 12 nymphs during the same instar and 12 nymphs at nymphal-adult moult. The morphology and longevity of the surviving nymphs were not affected. However, the number of adults formed was 16 percent less as compared to control.

Further, the average number of eggs laid by each female was 10.33 eggs less than that of control (Table - 44). Fertility of the eggs was reduced by 17.94 percent as compared to control. The fall in fecundity ($t = 1.02$, $p > 0.05$) and fertility ($t = 2.04$, $p > 0.05$) was statistically insignificant.

In the third test set, 2.0 ug 'Triol' was injected to each of 100 nymphs. Thereafter, 17 nymphs died before next moulting and 14 nymphs suffered mortality at nymphal-adult moulting. The morphology and longevity of the surviving nymphs remained unaffected. The number of adults formed was 23 percent less as compared to control (Table - 43). The average number of eggs laid by each affected female was 23.66 less than that of control (Table - 44) which was a insignificant loss ($t = 1.49$, $p > 0.05$). However, fertility of the eggs was significantly reduced by 29.31 percent as compared to control ($t = 2.62$, $p < 0.05$).

In the fourth group of 100 nymphs, each nymph was injected with 4.0 ug 'Triol'. It was found that 25 nymphs of the treated instar died at different intervals of time and 18 nymphs lost their life at nymphal-adult moult. Though the morphology of the remaining nymphs was unaffected, the longevity of the nymphs was shortened by 16-24 hours as compared to control. The formation of adults was 35 percent less as compared to control (Table - 43). The average number of eggs

laid by each affected female was 24.5 less as compared to control (Table - 44) which was statistically insignificant ($t = 1.38$, $p > 0.05$). Fertility of the eggs was significantly reduced by 35.02 percent as compared to control ($t = 2.75$, $p < 0.05$).

In the fifth group, each of the 100 nymphs was injected with 6.0 ug 'Triol'. Following the injection there was mortality in 39 nymphs of the treated instar and 18 nymphs at next moult. The morphology of the remaining nymphs was unaffected but longevity was abbreviated by 20-28 hours as compared to control. The number of adults formed from the treated nymphs was 49 percent less as compared to control (Table - 43). Each of the affected females, on average, laid 25.5 eggs less than that of control which was statistically insignificant loss ($t = 1.80$, $p > 0.05$). Fertility of the eggs was reduced by 62.22 percent as compared to control which was statistically highly significant ($t = 3.38$, $p < 0.05$).

(6) Effect of topical application of different doses of 'Triol' on 4th instar nymphs of Dysdercus cingulatus:

The effect of 'Triol', an analogue of ecdysone, was also studied on D. cingulatus. For this purpose different doses of this analogue as applied on S. litura and D. obliqua larvae (0.5, 1.0, 2.0, 4.0 and 6.0 ug) were topically applied

both on 4th and 5th instar nymphs and then observations were made on moulting, longevity, fecundity and fertility etc.

The present section deals with the observations following the treatment on 4th instar nymphs. Each dose was tested against 100 nymphs. For control insects were treated with acetone only. The procedure for the topical application is given under section "Materials and Methods".

In the first test of 100 nymphs, each nymph was topically applied with 0.5 ug 'Triol'. Then 8 treated nymphs died during the same instar and 8 other nymphs at next nymphal moult. Thereafter 7 nymphs died during the 5th stage and 6 nymphs could not survive at nymphal-adult moult. The morphology and longevity of the nymphs of either instar were unaffected. The adults emerged out of the treated nymphs were 20 percent less as compared to control (Table - 45). The fecundity and fertility were observed by pairing 6 females, which emerged from treated nymphs, with 6 males of untreated nymphal stock. The age of the females and males was similar. It was found that each affected female, on average, laid 5.16 eggs less than that of control (Table - 46) showing insignificant fall ($t = 0.75$, $p > 0.05$). Fertility of the eggs was also insignificantly reduced by 5.56 percent as compared to control ($t = 1.09$, $p > 0.05$).

In the second test, 100 nymphs were treated with 1.0 ug 'Triol' per nymph. It led to the mortality of 13 nymphs during the treated instar and 7 nymphs at next nymphal moulting. The morphology of treated and surviving nymphs was unaffected but their longevity was abbreviated by 12-15 hours as compared to control. Further, 7 nymphs died during the 5th instar and finally 4 nymphs could not survive at nymphal-adult moulting. However, the morphology and longevity of surviving 5th instar nymphs were unchanged. The number of adults emerged from the treated nymphs was 22 percent less as compared to control. Each of the affected females, on average, laid 15.33 eggs less than that of control (Table - 46). Reduction in fertility was 11.31 percent as compared to control. The reduction in fecundity ($t = 1.21$, $p > 0.05$) and fertility ($t = 1.66$, $p > 0.05$) was statistically insignificant.

In the third batch of 100 test nymphs, each nymph was treated with 2.0 ug 'Triol'. Thereafter, 13 nymphs died during the same instar and 10 nymphs at next nymphal moulting. The morphology of the surviving nymphs did not change but longevity was shortened by 15-18 hours as compared to control. Later, 6 nymphs died during the 5th stage and 10 nymphs suffered mortality at nymphal-adult moulting. The morphology and longevity of the surviving 5th instar nymphs were not affected. The number of adults emerged was 30 percent less

than that of control. Fecundity of the affected females was insignificantly reduced as each such female, on average, laid 26.0 eggs less than that of control ($t = 1.96$, $p > 0.05$). Fertility of eggs was insignificantly reduced by 11.09 percent (Table - 46) as compared to control ($t = 1.97$, $p > 0.05$).

In the fourth test 100 nymphs were tested with 4.0 ug 'Triol'/nymph. It was found that 15 nymphs died during the same stage and 12 nymphs at moulting to 5th instar. The morphology of the treated and surviving nymphs remained unaffected but longevity was shortened by 18-22 hours as compared to control. Later 10 nymphs died during the 5th instar and 7 nymphs could not survive at nymphal-adult moulting. The morphology and longevity of the 5th instar nymphs were not affected. The adults emerged were 35 percent less as compared to control (Table - 45). Each of the affected females, on average, laid 38.83 eggs less than that of control (Table-46) which was a insignificant fall ($t = 2.54$, $p > 0.05$). Reduction in fertility was 14.89 percent as compared to control which was also statistically insignificant ($t = 2.49$, $p > 0.05$).

In the fifth test, each of 100 nymphs was treated with 6.0 ug 'Triol'. The treatment resulted in the death of 21 nymphs during the same stage and 9 nymphs at next nymphal moult. The morphology of the treated 4th instar nymphs which survived was not affected. However, the longevity was abbre-

viated by 25-30 hours as compared to control. Later, 14 nymphs died during the 5th instar and finally 14 nymphs could not survive at nymphal-adult moulting. Likewise the morphology and longevity of the surviving 5th instar nymphs (moulted from treated 4th instar) were not changed. The adult emergence was reduced by 49 percent as compared to that of control (Table-45). Reduction in fecundity was significant ($t = 2.80$, $p<0.05$) as each affected female, on average, laid 60.16 eggs less than that of control (Table - 46). Fertility was also significantly reduced by 30.29 percent as compared to that of control ($t = 3.27$, $p<0.05$).

(H) Effect of topical application of different doses of 'Triol' on 5th instar nymphs of Dysdercus cingulatus:

The 5th instar nymphs of D. cingulatus were also topically treated with different doses of 'Triol' (0.5, 1.0, 2.0, 4.0 and 6.0 ug/nymph) which were the same as used in case of S. litura and D. poliqua. The age of these nymphs was 24 hours. Each dose was applied on 100 nymphs. For parallel control, insects were treated with acetone only as given in the section of "Materials and Methods".

In the first group of 100 insects, 0.5 ug 'Triol' was applied on each nymph. Following the treatment 12 nymphs suffered mortality during the same instar and 5 nymphs at

nymphal-adult moulting. The morphology and longevity of the treated instar which survived remained unaffected. The number of adults emerged from treated nymphs were 14 percent less as compared to that of control (Table - 47). Fecundity was insignificantly reduced ($t = 0.28$, $p > 0.05$) as each of the affected females, on average, laid 0.83 eggs less than that of control (Table - 48). Reduction in fertility of the eggs was 0.04 percent which was also statistically insignificant ($t = 0.30$, $p > 0.05$).

In the second group of 100 insects, each nymph was treated with 1.0 ug 'Triol'. It was found that 17 treated nymphs died during the treated instar where 7 similar nymphs suffered mortality at nymphal-adult moulting. However, the morphology and longevity of the survived treated nymphs did not change. Finally the adults emerged were 21 percent less as compared to that of control (Table - 47). Each of the affected females, on average, laid 28.66 eggs less than that of control (Table - 47). Fertility of the eggs was reduced by 17.18 percent as compared to that of control. The reduction in fecundity ($t = 1.48$, $p > 0.05$) and fertility ($t = 2.32$, $p > 0.05$) was statistically insignificant.

In the third group of 100 insects, each nymph was treated with 2.0 ug 'Triol'. Then, 18 nymphs of the treated instar died. Further, 11 nymphs suffered mortality at nymphal-

adult moulting. The morphology and longevity of the surviving treated nymphs remained unchanged. The number of adults emerged from the treated stock was 26 percent less as compared to that of control (Table - 47). Fecundity of the affected females was insignificantly reduced ($t = 1.52$, $p > 0.05$) as each such female, on average, laid 30.16 eggs less than that of control (Table - 48). Fertility was reduced by 22.56 percent as compared to control which was also statistically insignificant ($t = 2.29$, $p > 0.05$).

In the fourth set of 100 nymphs, each nymph was treated with 4.0 ug 'Triol'. It was found that 20 nymphs suffered mortality following this treatment. Later, 17 treated nymphs died at nymphal-adult moulting. The morphology and longevity of the surviving treated nymphs remained unaffected. The adult emergence was reduced by 34 percent as compared to that of control (Table - 47). Each of the affected females, on average, laid 34.66 eggs less than that of control (Table - 48) which was statistically insignificant ($t = 1.76$, $p > 0.05$). Fertility of the eggs was reduced by 28.77 percent as compared to that of control, which was also insignificant reduction ($t = 2.44$, $p > 0.05$).

In the fifth group, 100 nymphs were treated with 6.0 ug 'Triol'/nymph. Following the treatment 26 nymphs died during this instar and 14 nymphs could not survive at nymphal-adult

moulting. Though the morphology of the treated instar was unaffected, the longevity was shortened by 15-20 hours as compared to control. The adults emerged from treated nymphs were 37 percent less than that of control (Table - 47). Each of the affected females, on average, laid insignificantly 38.5 eggs less than that of control ($t = 1.94$, $p > 0.05$). Fertility of the eggs was reduced by 39.5 percent (Table - 48) as compared to control which was also insignificant change ($t = 2.56$, $p > 0.05$).

Table - 33: Showing nymphal mortality, adult emergence and total loss up to emergence following the injection of different doses of α-ecdysone to the 4th instar nymphs of Dysdercus cingulatus (Each dose was tested against 100 nymphs).

Dose/Nymph (ug)	Nymphal Mortality (Number)				Adult Emergence (Number)	Total loss up to emergence (%)
	4th instar	At next moult	5th instar	At nymphal-adult moult		
Control	3	-	2	-	95	5
0.5	3	1	2	2	92	8
1.0	5	2	3	1	89	11
2.0	7	2	2	2	87	13
4.0	9	5	5	2	79	21
6.0	15	3	4	5	73	27

Average of 5 replicates

Table - 34: Showing fecundity and fertility of females of Dysdercus cingulatus emerged from 4th instar nymphs injected with different doses of α-ecdysone.

Dose/nymph (ug)	No.of eggs laid (mean value) + S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	123.50 ± 7.75	119.00 ± 6.33	96.35
0.5	115.00 ± 9.89	107.16 ± 7.16	93.18
1.0	120.33 ± 6.45	111.66 ± 8.28	92.79
2.0	116.33 ± 11.70	104.83 ± 8.00	90.11
4.0	119.16 ± 8.85	111.00 ± 8.24	93.15
6.0	111.16 ± 11.59	99.33 ± 6.85	89.35

Table - 35: Showing nymphal mortality, adult emergence and total loss up to emergence following the injection of different doses of α-ecdysone to the 5th instar nymphs of Dysdercus cingulatus (Each dose was tested against 100 nymphs).

Dose/Nymph (ug)	Nymphal Mortality (Number)		Adult Emergence (Number)	Total loss up to emergence (%)
	5th instar	At nymphal-adult moult		
Control	3	3	94	6
0.5	6	5	89	11
1.0	5	4	91	9
2.0	12	11	77	23
4.0	11	10	79	21
6.0	28	9	63	37

Average of 5 replicates

Table - 36: Showing fecundity and fertility of females of Dysdercus cingulatus emerged from 5th instar nymphs injected with different doses of α-ecdysone.

Dose/nymph (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	127.50 ± 6.70	122.66 ± 5.28	96.20
0.5	124.16 ± 5.96	119.33 ± 5.21	96.10
1.0	110.5 ± 4.05	99.66 ± 4.26	90.19
2.0	104.50 ± 5.89	94.66* ± 4.15	90.52
4.0	95.66 ± 11.38	78.16* ± 9.42	81.70
6.0	77.66* ± 11.43	57.83* ± 5.27	74.46

* Significant at 5% level

Table - 37: Showing nymphal mortality, adult emergence and total loss up to adult emergence following the topical application of different doses of α-ecdysone to the 4th instar nymphs of *Dysdercus cingulatus* (Each dose was tested against 100 nymphs).

Dose/Nymph (ug)	Nymphal Mortality (Number)				Adult Emergence (Number)	Total loss up to emergence (%)
	4th instar	At next moult	5th instar	At nymphal-adult moult		
Control	6	1	2	1	90	10
0.5	6	4	3	2	85	15
1.0	9	5	6	5	75	25
2.0	12	4	5	6	73	27
4.0	15	4	8	4	69	31
6.0	20	6	10	5	59	41

Average of 5 replicates

Table - 38: Showing fecundity and fertility of females of *Dysdercus cingulatus* emerged from 4th instar nymphs topically treated with α-ecdysone.

Dose/nymph (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	139.83 ± 14.00	128.33 ± 13.05	91.77
0.5	134.50 ± 20.37	128.16 ± 17.91	95.28
1.0	133.83 ± 16.37	125.16 ± 17.30	93.52
2.0	133.33 ± 6.31	112.66 ± 8.48	84.49
4.0	126.33 ± 18.20	110.66 ± 19.10	87.59
6.0	121.83 ± 17.05	99.33 ± 12.39	81.53

Table - 33: Showing nymphal mortality, adult emergence and total loss up to adult emergence following the topical application of different doses of α-ecdysone to the 5th instar nymphs of Dysdercus cingulatus (Each dose was tested against 100 nymphs).

Dose/Nymph (ug)	Nymphal Mortality (Number)		Adult Emergence (Number)	Total loss up to emergence (%)
	5th Instar	At nymphal-adult moult		
Control	5	-	95	5
0.5	8	3	89	11
1.0	14	5	81	19
2.0	23	4	73	27
4.0	27	4	69	31
6.0	30	6	64	36

Average of 5 replicates

Table - 40: Showing fecundity and fertility of females of Dysdercus cingulatus emerged from 5th instar nymphs topically treated with α-ecdysone.

Dose/nymph (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	145.83 ± 10.76	139.83 ± 11.74	95.98
0.5	137.83 ± 11.95	120.66 ± 8.63	87.54
1.0	130.83 ± 13.66	104.50 ± 12.74	79.87
2.0	127.00 ± 7.34	99.00 ± 6.22	77.95
4.0	113.00 ± 5.34	76.66* ± 7.26	67.84
6.0	110.50 ± 8.00	71.5* ± 11.91	64.70

* Significant at 5% level

Table - 41: Showing nymphal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of 'Triol' to the 4th instar nymphs of *Dysdercus cingulatus* (Each dose was tested against 100 nymphs).

Dose/Nymph (ug)	Nymphal Mortality (Number)				Adult Emergence (Number)	Total loss up to emergence (%)
	4th instar	At next moult	5th instar	At nymphal-adult moult		
Control	5	-	7	3	85	15
0.5	11	7	9	6	67	33
1.0	14	8	9	11	58	42
2.0	19	7	13	16	45	55
4.0	23	9	17	16	35	65
6.0	29	13	18	19	21	79

Average of 5 replicates

Table - 42: Showing fecundity and fertility of females of Dysdercus cingulatus emerged from 4th instar nymphs injected with different doses of 'Triol'.

Dose/nymph (ug)	No. of eggs laid (mean value) ± S.E.	No. of eggs hatched (mean value) ± S.E.	% hatching
Control	114.83 ± 8.02	112.00 ± 8.01	97.53
0.5	113.33 ± 9.39	108.00 ± 8.14	95.29
1.0	108.33 ± 8.61	93.50 ± 5.91	86.31
2.0	112.83 ± 7.04	90.50 ± 4.77	80.20
4.0	94.00 ± 11.38	63.83* ± 4.82	67.90
6.0	90.83 ± 9.18	52.33* ± 6.50	57.61

* Significant at 5% level

Table - 43: Showing nymphal mortality, adult emergence and total loss up to adult emergence following the injection of different doses of 'Triol' to the 5th instar nymphs of Dysdercus cingulatus (Each dose was tested against 100 nymphs).

Dose/Nymph (ug)	Nymphal Mortality (Number)		Adult Emergence (Number)	Total loss up to emergence (%)
	5th instar	At nymphal-adult moult		
Control	5	3	92	8
0.5	11	8	81	19
1.0	12	12	76	24
2.0	17	14	69	31
4.0	25	18	57	43
6.0	39	18	43	57

Average of 5 replicates

Table - 44: Showing fecundity and fertility of females of Dysdercus cingulatus emerged from 5th instar nymphs injected with different doses of 'Triol'.

Dose/nymph (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	120.16 ± 11.08	112.00 ± 7.40	93.20
0.5	107.00 ± 14.95	[illegible] ± 10.37	86.13
1.0	[illegible] ± 13.08	[illegible] ± 9.84	75.28
2.0	96.50 ± 14.99	61.66* ± 10.63	63.89
4.0	98.66 ± 12.73	56.66* ± 10.65	58.18
6.0	94.66 ± 8.06	[illegible] ± 5.34	30.98

* Significant at 5% level

Table - 45: Showing nymphal mortality, adult emergence and total loss up to adult emergence following the topical application of different doses of 'Triol' to the 4th instar nymphs of Dysdercus cingulatus (Each dose was tested against 100 nymphs).

Dose/Nymph (ug)	Nymphal Mortality (Number)				Adult Emergence (Number)	Total loss up to emergence (%)
	4th instar	At next moult	5th instar	At nymphal-adult moult		
Control	5	1	3	-	91	9
0.5	8	8	7	6	71	29
1.0	13	7	7	4	69	31
2.0	13	10	6	10	61	39
4.0	15	12	10	7	56	44
6.0	21	9	14	14	42	58

Average of 5 replicates

Table - 46: Showing fecundity and fertility of females of Dysdercus cingulatus emerged from 4th instar nymphs topically treated with 'Triol'.

Dose/nymph (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	127.16 ± 7.86	116.33 ± 10.62	91.48
0.5	122.00 ± 14.41	104.83 ± 12.81	85.92
1.0	111.83 ± 17.52	89.66 ± 13.00	80.17
2.0	101.16 ± 8.68	81.33 ± 11.47	80.39
4.0	88.33 ± 6.86	67.66 ± 8.59	76.59
6.0	67.00* ± 10.93	41.00* ± 6.62	61.19

* Significant at 5% level

Table - 47: Showing nymphal mortality, adult emergence and total loss up to adult emergence following the topical application of different doses of 'Triol' to the 5th instar nymphs of Dysdercus cingulatus (Each dose was tested against 100 nymphs).

Dose/Nymph (ug)	Nymphal Mortality (Number)		Adult Emergence (Number)	Total loss up to emergence (%)
	5th instar	At nymphal-adult moult		
Control	3	-	97	3
0.5	12	5	83	17
1.0	17	7	76	24
2.0	18	11	71	29
4.0	20	17	63	37
6.0	26	14	60	40

Average of 5 replicates

V. DISCUSSION

The administration of exogenous hormone to insects often proves difficult. Ingestion (Fourche, 1967) and cutaneous application (Horn, 1971) provide the easiest means, but in each case uptake may be highly variable from animal to animal. Further, in some species the absorption of ecdysones (moulting hormones) from the digestive tract may be limited (Staal, 1967) and thus its relative action on the biology of the treated species may differ markedly from that administered by other methods (Robbins et al., 1968). Administration through injection has also been preferred by some workers so that the effect of varying dosage could be assessed accurately.

In the present investigations natural ecdysones (α- and β-isomers) and an analogue, 'Triol', were applied on the larvae of Spodoptera litura as well as Diacrisia obliqua and nymphs of Dysdercus cingulatus to observe their effects on growth and reproduction of these insects. The methods employed were injection, ingestion or topical.

Thus, 5th instar larvae of S. litura and D. obliqua were injected with different doses (0.5, 1.0, 2.0, 4.0 and 6.0 ug/larva) of β- and α-ecdysones (natural ecdysones) respectively whereas 4th instar nymphs of D. cingulatus were injected with α-ecdysone. In all these species larval/nymphal mortality

Table - 48: Showing fecundity and fertility of females of Dysdercus cingulatus emerged from 5th instar nymphs topically treated with 'Triol'.

Dose/nymph (ug)	No.of eggs laid (mean value) ± S.E.	No.of eggs hatched (mean value) ± S.E.	% hatching
Control	131.66 ± 13.33	123.16 ± 11.30	93.54
0.5	130.83 ± 10.87	122.33 ± 10.19	93.50
1.0	103.00 ± 18.48	78.66 ± 10.65	76.36
2.0	101.50 ± 18.49	69.00 ± 13.88	67.98
4.0	97.00 ± 13.91	62.83 ± 13.36	64.77
6.0	93.16 ± 11.56	50.33 ± 15.89	54.04

was observed at all doses and it relatively increased with the stronger doses of the respective hormones. Comparatively maximum larval mortality was found in the 5th instar larvae of S. litura (42%) following the injection of the strongest dose (6.0 ug) of β-ecdysone (Table - 1). Similarly, following the injection of above mentioned doses to last-instar larvae (6th instar) of S. litura and D. obliqua and last-instar nymphs (5th instar) of D. cingulatus, the larval/nymphal mortality increased with successive higher doses of both ecdysones. And, again, the larvae of S. litura showed highest mortality (45%) than those of the other two species. Further, it is also clear that the injection of β-ecdysone to the 6th instar larvae of S. litura is more fatal than that of 5th instar (Fig. 5).

Further, when 5th instar larvae of both S. litura and D. obliqua were fed with the above mentioned doses of β- and ∝-ecdysone respectively, it was found that the mortality in the larvae of D. obliqua fed with ∝-ecdysone was higher (53%) at 6.0 ug dose/larva (Table - 21) than that in S. litura larvae (34%)(Table - 5) which ingested similar dose of β-ecdysone. However, larval mortality increased to 60% (as compared to control) at 6.0 ug β-ecdysone per larva, when the larvae were fed this hormone at the 6th instar. On the other hand, ingestion of 6.0 ug ∝-ecdysone per larva by 6th instar of D. obliqua causes only 28% larval mortality. It thus indicates that ∝-ecdysone is

either partially metabolised to non-toxic substance or the 6th instar larvae of D. obliqua physiologically resist the toxic action of this hormone. It is clear that in S. litura ingestion of 6.0 ug β-ecdysone per larva is lethal to 6th instar larvae. As regards the action of 'Triol' which was applied in similar doses as those of β-ecdysone or ∝-ecdysone to the larvae of 5th and 6th instar of S. litura and D. obliqua, the larval mortality was highest (100%) in the 6th instar larvae of S. litura which were injected with 4.0 ug and 6.0 ug 'Triol' larva whereas the injection of latter dose to 6th instar larvae of D. obliqua causes only 43% mortality. Similarly, on feeding 'Triol' doses to the larvae of both (5th & 6th) instars of S. litura and D. obliqua the larvae of the former species showed higher mortality than that of the latter species and maximum mortality was 58% following the ingestion of 6.0 ug dose by the 6th instar larvae of S. litura. As compared to this, D. obliqua larvae are less susceptible by even the strongest dose (6.0 ug)(Fig. 8). Therefore, 'Triol' is more lethal to S. litura than D. obliqua larvae.

In D. cingulatus when ∝-ecdysone and 'Triol' were topically applied on 4th and 5th instar nymphs and the doses were the same as used in case of S. litura and D. obliqua there was nymphal mortality at all doses of both the hormones and it increased with successive higher doses (Fig. 11). In this

species maximum nymphal mortality was due to 'Triol' application rather than that of α-ecdysone. Further, this data (49%) was obtained on 4th instar nymphs following the treatment. Therefore, it is clear that D. cingulatus is much susceptible to the 'Triol' at earlier stage of larval growth than that of S. litura and D. obliqua (6th instar). It is well known fact that the titre of moulting hormone in the haemolymph of developmental stages of insects gradually falls as metamorphosis approaches. Thus in the present investigation, at advanced stages of larval growth i.e., 5th and 6th instars of S. litura and D. obliqua and 4th and 5th instars of D. cingulatus the titre of the moulting hormone is enhanced in the haemolymph following the injection, feeding or topical application of their different doses. It is also obvious that the increase in moulting hormone concentration in the haemolymph at advanced growth stage may lead to abnormality in the treated insects by disturbing its homeostasis and one of the abnormal conditions appears to be mortality due to toxicity. Kubo et al. (1981) explained that oral application of 20-hydroxyecdysone and cyasterone on silkworm and the pink boll worm caused ecdysis inhibition through successive moults until death occurred and the effect was overtly hormonal rather than simple toxicity. However, the toxicity of various ecdysones and their analogues leading to larval mortality has also been reported in many other insects e.g., Musca domestica, Tribolium confusum, Aedes aegypti

and Blatella germanica (Robbins et al., 1970; Singh & Russell, 1980; Singh et al., 1982). Aside, Singh & Russell (1980) emphasized that in ingestion method the toxicity of the ecdysteroids depends upon the diet composition. According to them 20-hydroxyecdysone added to the amino acid diet of housefly caused lower mortality than in the casein diet. Mansingh (1976) from his observations on Rhodnius prolixus following the ingestion of β-ecdysone (ecdysterone) suggested that the larval mortality could have been the result of some physiological derangement.

The topical application of different doses of both α-ecdysone and 'Triol' on the 4th and 5th instar nymphs of D. cingulatus causes nymphal mortality which is more significant in 4th instar (Fig. 11). It is an important record in the light of the earlier explanation of Robbins et al. (1968) and Slama et al. (1974) that, in general, ecdysteroids are not able to penetrate the insect cuticle easily and no effects are observed if they are applied topically in aqueous solution. In the present investigation on D. cingulatus, the ecdysteroids were applied in acetone solution. Thus it is likely that some hormone may penetrate the cuticle through acetone as solvent and especially it may be more possible in less sclerotized cuticle of 4th instar nymphs as compared to relatively much thicker cuticle of 5th instar. However, the mode of action of topically

applied hormones through the cuticular sensillae cannot be ruled out.

The application of exogenous moulting hormone (α-, β- and 'Triol') was also found to cause an adverse effect on the larval/nymphal longevity of the present experimental insects. In S. litura, the injection of only strongest dose (6.0 ug) of β-ecdysone caused the reduction in the longevity of 6th instar larvae by 20-24 hours which were treated at this stage. The lower doses (2.0 ug and 4.0 ug) had no effect on the longevity of this instar. However, the longevity of the 5th instar larvae remained unaffected following the injection of any of these doses of β-ecdysone. When 6.0 ug β-ecdysone was fed to the larvae of either 5th or 6th instar S. litura, the longevity of these instars was abbreviated by 10-15 hours and 24-36 hours respectively. Ingestion of lower doses of this hormone did not affect the longevity of either instar except in case of 6th instar larvae which was shortened by 20-24 hours following the ingestion of 4.0 ug dose. The larval duration of both 5th and 6th instar larvae was, however, usually abbreviated following the injection or feeding the 4.0 ug or 6.0 ug doses of 'Triol'. Injection of 'Triol' caused rather more adverse effect on the longevity than by feeding. It is clear by the fact that the maximum reduction in the larval longevity of S. litura (30-40 hours) was found following the injection of 6.0 ug

hormone to the 6th instar larvae. Thus, in this species larval longevity is more appreciably reduced by an analogue of moulting hormone than by natural hormone.

In *D. obliqua* injection of α-ecdysone (2.0, 4.0 and 6.0 ug/larva) to the 5th instar larvae reduced the larval longevity but when these doses were injected to 6th instar larvae, their longevity was unaffected. However, the injection of the corresponding doses of 'Triol' to the 5th or 6th instar larvae of *D. obliqua* did not affect their longevity. On the other hand, the ingestion of higher doses (4.0 and 6.0 ug) of 'Triol' by either 5th or 6th instar larvae shortened the larval duration and the strongest dose of this hormone reduced the larval longevity of 5th instar (30-34 hours) more than that of 6th instar treated larvae (22-26 hours). It is, therefore, obvious that maximum reduction in larval longevity of both *S. litura* (30-40 hours) and *D. obliqua* (30-40 hours) was caused by 'Triol' (an analogue of moulting hormone) rather than β-ecdysone and α-ecdysone (natural ecdysones) at 6.0 ug dose. However, this action of 'Triol' is through injection in *S. litura* and through feeding of the same dose in *D. obliqua*. It appears that following the injection of hormone in the haemocoel of the larvae of *D. obliqua* the hormone alongwith blood oozes out as a result of reflex bleeding which may be possible and operative due to densely occurrence of sensory hairs on the

entire cuticle of these larvae as compared to that of S. litura larvae. Therefore, quantity of injected hormone in Diacrisia larvae may mix with the blood only partially. In D. cingulatus injection of ∝-ecdysone to 4th as well as 5th instar nymphs did not affect the nymphal longevity of either instar. Thus D. cingulatus differs from that of D. obliqua. In the former species at the advanced growth stage addition of ∝-ecdysone has no effect on the longevity of the instars whereas in the latter species the increased titre of this hormone abbreviates the longevity of the treated instar. The injection of 'Triol' to 4th instar nymphs of this bug also had no effect on their longevity but the nymphal duration of 5th instar nymphs treated with this hormone was shortened by 16-24 hours and 20-28 hours at 4.0 ug and 6.0 ug dose respectively whereas the lower doses produced no effect. The topical application of ∝-ecdysone had no effect on the nymphal longevity of 4th instar whereas similar application on the 5th instar nymphs adversely affected their longevity. However, topical application of the present doses of 'Triol' affected the nymphal duration of both 4th and 5th instar nymphs. Further, the reduction in the nymphal duration of 5th instar nymphs following the topical application of ∝-ecdysone and that of 4th instar nymphs treated with 'Triol' were same (i.e., 25-30 hours) at 6.0 ug dose. Topical treatment of 'Triol' on 4th instar nymphs was effective in reducing the longevity even at lower doses (2.0 ug and 4.0 ug) whereas

in case of 5th instar nymphs the reduction occurred only by 6.0 ug dose.

It is clear that the administration of exogenous ecdysones increases the concentration of the hormone in the circulating blood which consequently accelerates the moulting process. It is also evident that in these species the ecdysone analogue ('Triol') induces moulting processes comparatively much earlier than the natural ecdysones (α- and β-ecdysones).

Reduction in the larval/nymphal duration following the treatment with ecdysones has been found in other insects also. The 4th instar larvae of Epilachna varivestis attempted ecdysis within 4-5 days compared with the 7-day period for the controls following the treatment with 1000 ppm of 22,25-bisdeoxyecdysone (Walker & Thompson, 1973). Similarly, 20-hydroxyecdysone at 100 ppm concentration given to housefly larvae in amino acid diet showed an ET_{50} (time taken for 50% of the larvae to pupate) of 11.4 days compared to 13.9 days for the control (Singh & Russell, 1980).

In all orders of Exopterygotes (e.g., Orthoptera, Dictyoptera & Hemiptera etc.) and also in that of many Endopterygotes (e.g. Diptera, Lepidoptera and Coleoptera etc.) so far investigated, supernumerary larval or pupal instars developed by transplanting active endocrine glands or applying

juvenile hormone or ecdysones and their analogues (Pflugfelder, 1958; Novak, 1966; Slama, 1971 and Zdarek & Slama, 1972). However, in the larvae of higher Diptera such as those of Cyclorrhapha, injection of 20 ug ecdysterone per larva produced supernumerary larval instars but the application of 5 ug dose could not produce such instars. The present data show that when 6th instar larvae of S. litura were injected or fed with 4.0 ug and 6.0 ug β-ecdysone they attempted to moult to extra instar or supernumerary larvae (Fig. 1). Following the injection of 4.0 ug β-ecdysone to each of the 6th instar larvae, among the surviving larvae 10.38% attempted to moult to extra instar but failed and died in the process. However, at 6.0 ug dose 17.14% of the surviving larvae made such an attempt but among them 16.66% successfully transformed into supernumerary larvae (Table - 3) whereas the rest failed and died in the process. Similarly the ingestion of 4.0 ug and 6.0 ug β-ecdysone by the 6th instar larvae of S. litura resulted in the formation of supernumerary larvae. Among the larvae that survived following the ingestion of 4.0 ug β-ecdysone, 8.45% attempted to moult to extra instar but among them 16.66% successfully transformed into supernumerary larvae and the rest failed and died. Further at 6.0 ug dose, 23.07% of the surviving larvae attempted to moult to extra instar. Out of such larvae 33.33% became able to transform into supernumerary larvae (Table - 7). On the other hand, the application (injection or ingestion) of 'Triol'

doses on the larvae of S. litura did not result in the formation of supernumerary larvae. It was further evident that supernumerary larval or nymphal formation was also absent in D. obliqua and D. cingulatus following the treatment with both, natural ecdysone (α-ecdysone) or ecdysone analogue ('Triol').

It is well known fact that the increase in juvenile hormone concentration in the fully grown larvae prevents metamorphosis and the larvae moult to a supernumerary larval instar. Similar effect seen by the administration of 4.0 and 6.0 ug β-ecdysone on the 6th instar larvae of S. litura confirms that enhanced titre of this moulting hormone in the haemolymph accelerates moulting process without inhibiting the effect of diminishing juvenile hormone at this stage of exogenous β-ecdysone may induce further secretion of juvenile hormone which maintains the juvenile character. However, application of β-ecdysone on the 5th instar larvae of S. litura had no further effect to produce extra-instar larvae after the 6th instar. Therefore, it is possible that production of supernumerary larvae is a result of increase in β-hormone concentration of the blood at a stage when larval growth is in advanced condition. Walker and Thompson (1973) also reported that the 2nd instar larvae of Epilachna varivestis confined with plants dipped into solution containing 1000 ppm of 22,25-bisdeoxyecdysone resulted in the formation of a supernumerary instar with extended wing

pads. Similarly high doses of ecdysterone (20 ug/larva) injected just after ecdysis or in starved larvae caused a precocious moult and the formation of supernumerary larvae in Calliphora vomitoria and Sarcophaga argyrostoma. Further, in the present study the absence of formation of such an extra instar in D. obliqua and D. cingulatus shows that there exists a wide variation in the susceptibility of different insects to these compounds.

In S. litura larvae treated with either β-ecdysone or 'Triol', there was no larval malformation except supernumerary larvae. But in D. obliqua malformation in the larvae treated with ∝-ecdysone as well as 'Triol' was found rather frequently, which were represented as neotenic forms, precociously moulted forms, larvae which failed to cast off their exuviae and also larval-pupal intermediates (Fig. 2 & 3). Such instances of larval malformations were prevalent more in case of the larvae treated with ∝-ecdysone either by injections or ingestion rather than in 'Triol' treated larvae. This observation is in conformation with the opinion of Robbins et al. (1970) that the natural ecdysones are more active than the analogues in the moulting assay. It is quite contrasting fact that there was development of larval-pupal intermediates following the injection of ∝-ecdysone or 'Triol' by either instar of D. obliqua but not following the ingestion of these hormones. The injection of all the doses of ∝-ecdysone to both 5th and 6th instar

larvae of D. obliqua resulted in the formation of such intermediates (Tables- 17 & 19). On the other hand, such effect was also seen following the injection of all doses of 'Triol' to the 5th instar larvae but in the 6th instar larvae only the higher doses (4.0 and 6.0 ug) could produce such intermediates. Both α-ecdysone and 'Triol' injected to 5th instar larvae caused higher incidence of larval-pupal intermediates (17.14% and 18.51% respectively) than that of 6th instar (7.89% and 8.92% respectively) larvae. The larval-pupal intermediates were reported in many other groups of insects. In case of Calliphora sp. the injection of 20 ug ecdysterone/larva a few hours after ecdysis led to the formation of larval pupal intermediates displaying the mosaic of tanned areas in the cuticle (Zdarek & Slama, 1972).

There was occasional occurrence of malformed pupae following the injection of β-ecdysone to either 5th or 6th instar larvae of S. litura whereas the injection of 'Triol' to these larvae caused no malformation at all. Thus, it happened only when 6th instar larvae were injected with 4.0 ug and 6.0 ug β-ecdysone (2.89% and 5.17% respectively). However, the ingestion of these hormones by either instar of S. litura showed higher incidence of malformed pupae. The maximum occurrence of such pupae was observed following the ingestion of 6.0 ug β-ecdysone/larva by the 5th and 6th instar larvae

(20.78% and 26.65% respectively). But 'Triol' ingestion produced very small percentage of abnormal pupae (2.77%) and that was only by the strongest dose (6.0 ug) eaten by 5th instar larvae whereas in case of 6th instar larvae even lower doses of this analogue also produced such pupae though the incidence was much lower (6% at 6.0 ug) as compared to β-ecdysone. In D. obliqua, 6th instar larvae injected with either α-ecdysone or 'Triol' did not lead to the formation of abnormal pupae. But both the hormones when injected to 5th instar larvae resulted in the frequent formation of such pupae by almost all the doses (2.0 ug, 4.0 ug and 6.0 ug). Thus the injection of 6.0 ug α-ecdysone to 5th instar larvae of D. obliqua caused 17.24% malformed pupae as compared to 9.09% obtained following the injection of similar dose of 'Triol' to the larvae of same instar.

As regards the effect of ingestion of these hormones on pupal malformation, α-ecdysone had no such effect either after eating by 5th instar or 6th instar larvae. On the other hand, ingestion of 'Triol' in 4.0 ug and 6.0 ug doses by the 5th instar larvae and all the doses by 6th instar larvae resulted in the formation of abnormal pupae. Thus after the intake of 6.0 ug 'Triol' per larva of 5th and 6th instar pupal malformation was 10% and 22.05% respectively. It is, therefore, concluded that the incidence of pupal malformation in both S. litura and D. obliqua was higher following the ingestion rather than

injection of these hormones probably due to the fact that in injection method some amount of haemolymph along with ecdysone oozes out as a result of reflex bleeding. It is further evident that in S. litura, although application of 'Triol' by injection or ingestion to either 5th or 6th instar larvae could not produce extra larval instar, the same ecdysone analogue proved to be effective in the development of abnormal pupae.

It can, therefore, be suggested that the appearance of abnormalities in pupae by exogenous ecdysones is caused by the disruption in the conditions which programme the processes leading to pupal formation.

The number of adults emerged from the larvae of S. litura and D. obliqua as well as nymphs of D. cingulatus treated with the present hormones was also found to be considerably reduced. In. S. litura all the doses of both the hormones (β-ecdysone and 'Triol') were effective in reducing the yield of adults. The number of adults that emerged following the injection of 6.0 ug β-ecdysone per larva of the 5th and 6th instar S. litura was reduced by 42% and 45% respectively. But injection of 'Triol' was comparatively more effective to reduce adult emergence than β-ecdysone. Thus the number of adults emerged from the 5th instar larvae treated with 6.0 ug 'Triol' was reduced by 78% whereas 4.0 ug and 6.0 ug doses of the same analogue when injected to 6th instar larvae eliminated the yields

of adults completely (100%). However, the ingestion of even strongest dose (6.0 ug) of β-ecdysone by the 5th and 6th instar larvae of S. litura reduced the number of adults that emerged from these larvae only by 34% and 60% respectively. Ingestion of 'Triol' also caused depletion in adult number by 83% and 58% when 6.0 ug dose of this hormone was fed to each larva of 5th and 6th instars respectively. Therefore, in S. litura it is clear that generally injection of 'Triol' to the advanced stage larvae consequently leads to more significant fall in adult emergence than by feeding this hormone.

In D. obliqua, application of α-ecdysone as well as 'Triol' affected adult emergence which was also proportionately related with the dose concentration. The injection of highest dose of α-ecdysone (6.0 ug/larva) to the 5th and 6th instar larvae lowered the formation of adults by 36% and 20% respectively whereas following the injection of same dose of 'Triol', the reduction in the adult number was 37% and 43% respectively. Similarly following feeding of the strongest dose of α-ecdysone to the larvae of D. obliqua the number of adults formed was reduced by 53% and 28% whereas that of 'Triol' reduction was 85% and 38% respectively. It contributes to the fact that α-ecdysone (natural ecdysone) injection to the larvae of D. obliqua is not equally effective in reducing adult emergence as compared to that of β-ecdysone (also a natural ecdysone) on

S. litura. Further, in the former species feeding of α-ecdysone leads to more fall in adult emergence as compared to injection of the same dose. Again, the reason for this observation may be that after injection of any dose in D. obliqua larvae, some injected material may ooze out as a result of reflex bleeding.

The sum effect following the application of α-ecdysone as well as 'Triol' on the nymphs of advanced instars of D. cingulatus, also resulted in decreasing the adult formation by both injection and topical application. Further, this change was found by all doses and it relatively increased with the higher doses of these hormones. 'Triol' applied by either injection or topically was more effective in this regard than α-ecdysone. The number of adults emerged from 4th and 5th instar nymphs injected with 6.0 ug/nymph of α-ecdysone was reduced by 22% and 31% respectively whereas the injection of same dose of 'Triol' (analogue) dropped adult number by 64% and 49% respectively. In comparison to this data following the topical application of strongest dose of α-ecdysone to both 4th and 5th instar nymphs, adult emergence suffered by 31% in each case whereas it was respectively 49% and 37% when the similar dose of 'Triol' was topically applied on these nymphs. Similar reduction in the yield of adults at 3 ppm concentration

and complete elimination of the formation of adults at 10 ppm concentration occurred in case of Anthonomous grandis when a trihydroxy 6-keto steroid was added to the larval diet (Earle et al., 1970). On the basis of the present data it can be suggested that for controlling the adult emergence of S. litura D. obliqua and D. cingulatus,'Triol' (analogue) may be preferred over natural ecdysones (α-or β-ecdysone).

There was no malformation in the emerged adults of S. litura and D. cingulatus following the administration of the present ecdysones on the respective larval/nymphal forms. But in D. obliqua malformation in the adults emerged from the larvae treated with either hormone did occur (Fig. 4). The malformed adults generally had folded fore-wings or hind-wings or both. All the doses (0.5, 1.0, 2.0, 4.0 and 6.0 ug per larva) of 'Triol' given by either injection or feeding produced such malformed adults. As regards α-ecdysone given through injection malformation in adults of D. obliqua occurred both at lower and higher doses whereas by feeding only the strongest dose (6.0 ug) caused such malformation. Thus the maximum malformation (44.44%) appeared in the adults emerged from the 5th instar larvae injected with 6.0 ug 'Triol' (Table - 25).

Both fecundity and fertility of S. litura, D. obliqua and D. cingulatus are adversely affected by the application of exogenous moulting hormones (α-ecdysone, β-ecdysone and

analogue, 'Triol').

In S. litura, following the application of both β-ecdysone and 'Triol' production of eggs was effectively inhibited which was relatively higher with increasing doses of the hormones (Fig. 6). There were 70.09% and 81.2% reductions in the number of eggs laid by the females emerged from the 5th and 6th instars larvae respectively which were injected with strongest dose (6.0 ug) of β-ecdysone. But after injecting the same dose of 'Triol' to the 5th instar larvae the emerged females were highly infecund and out of 6 females only 1 female was able to lay eggs (76 eggs). Since there was 100% mortality up to adult emergence following the injection of 4.0 ug and 6.0 ug doses of 'Triol' on 6th instar larvae (Table - 11) fecundity could not be assessed on females which would have emerged from such treated larvae. On the other hand following the ingestion of both β-ecdysone and 'Triol' in the similar doses to the larvae of either instar of S. litura suppression in the fecundity by the former (60.54% and 89.95%) was higher than by the latter (18.76% and 17.48%).

The fecundity of D. obliqua females was also adversely affected following the application of α-ecdysone and 'Triol' on its larvae (Fig. 9). In this species natural ecdysone (α-ecdysone) was usually more effective inhibitor of fecundity than that of the analogue (Triol'). The fecundity of the

females emerged from the 5th and 6th instar larvae injected with 6.0 ug ∝-ecdysone was reduced by 20.24% and 34.59% respectively whereas injection of similar dose of 'Triol' decreased the fecundity by 65.01% and 75.88%. However, by ingestion of ∝-ecdysone or 'Triol' in similar doses by the larvae of either instar was not as effective as injection on the fecundity. It is evident by the fact that following the ingestion of 6.0 ug ∝-ecdysone per larva of 5th and 6th instars the fecundity of emerged females decreased by 27.42% and 31.68% respectively. Similarly, following the ingestion of the same dose of 'Triol' by these larvae later on affected the fecundity of the females which were respectively 21.70% and 48.92% less than those of the control (Table - 14 & Table - 16).

In D. cingulatus females, emerged from 4th and 5th instar nymphs which were treated with ∝-ecdysone and 'Triol' by injection or topical application, fecundity was affected (Fig. 12). Thus by injecting strongest dose (i.e., 6.0 ug) of ∝-ecdysone to the nymphs of 4th and 5th instars egg production dropped by 9.99% and 39.10% respectively (Table - 34 and Table - 36) whereas injection of similar dose of 'Triol' decreased the fecundity of the emerged females by 20.91% and 21.23% respectively indicating that 'Triol' had almost similar effect irrespective of the stage of application. But ∝-ecdysone application on the advanced stage of growth reduced fecundity more than that of earlier stage. The topical application of ∝-ecdysone as well as 'Triol' on the

4th and 5th instar also reduced the fecundity of their respective females. Maximum effect was that of 6.0 ug dose of both the ecdysones. However, 'Triol' produced higher infecundity than that of α-ecdysone (Fig. 12). On D. cingulatus, Jalaja et al. (1976) have also studied the effect of ecdystrone (β-ecdysone). But they injected 2.0 ug and 4.0 ug hormone per female (one day old) on four consecutive days and they found 50% reduction in the fecundity of the females at 4.0 ug dose. In comparison to this in the present investigation on D. cingulatus, α-ecdysone and 'Triol' were applied on the nymphal stages (4th and 5th) and both the hormones by either method of application, injection or topical, significantly reduced the fecundity of the females which emerged from the treated nymphs. Maximum reduction in the fecundity (47.32%) of the females emerging from these nymphs was obtained by the topical treatment of 6.0 ug 'Triol' on 4th instar nymphs whereas α-ecdysone was less effective. It is, therefore, clear that 4.0 ug β-ecdysone used by Jalaja et al. on D. cingulatus females on successive four days caused the reduction (50%) in fecundity which is equal to that in the present study (47.32%) when single dose of 6.0 ug 'Triol' was topically applied on the 4th instar nymphs (Table - 46).

Like fecundity, fertility of the eggs laid by the females emerged from the larvae of S. litura and D. oblicua and nymphs of D. cingulatus which were treated with the selected ecdysones

in the present investigation was also reduced and such fall was relatively higher with stronger doses of the ecdysones. Thus following the injection of 6.0 ug β-ecdysone per larva there was 60.6% and 70.15% drop in the fertility of the eggs of the females emerged from the 5th and 6th instar larvae of S. litura. On the other hand, the same dose of 'Triol' (6.0 ug) injected to the 5th instar larvae of S. litura lead to high infecundity and only one out of 6 females laid eggs (76 eggs) in which 45 eggs were viable. Since the injection of 4.0 ug and 6.0 ug 'Triol' per larva to the 6th instar larvae completely eliminated the formation of adults so fertility could not be estimated. The present data substantiate that ingestion of the same dose of either β-ecdysone or 'Triol' to the larvae of the respective instars of S. litura causes greater effect on the fertility of the emerged females i.e., 32.92% and 60.33% respectively by β-ecdysone and 50.58% and 78.76% respectively by 'Triol' (Fig. 7). Further, 'Triol' was more effective in this respect. Like S. litura, the fertility of the eggs laid by the D. obliqua females was also adversely affected by the application of α-ecdysone and 'Triol' (Fig. 10). However, α-ecdysone caused less reduction in the fertility of D. obliqua than β-ecdysone in S. litura. Further, there was no difference in the fertility of the females of D. obliqua whether these emerged following the injection of even strongest dose (6.0 ug α-ecdysone) to either 5th instar or 6th instar larvae (8.26%

and 8.60%) respectively. Similarly, there was no difference in the fertility of the females which emerged from either 5th or 6th instar larvae fed with that dose of α-ecdysone.

As in case of S. litura, 'Triol' was more effective in reducing the fertility of D. obliqua than the natural hormone (α-ecdysone in this species). The injection of this analogue caused 40.77% and 47.48% decrease in the fertility of the females emerged from 5th and 6th instar larvae respectively. However, by feeding the strongest dose to 5th instar larvae there was 47.25% reduction in the fertility of the females but feeding the same dose to 6th instar resulted in less fall (12.51%).

In D. cingulatus also application of exogenous moulting hormones on the advanced nymphal stages adversely affected the fertility of the females. However, in this respect 'Triol' was more effective than α-ecdysone when applied either by injection or topically. It is evident by the data given in Fig. 13. There was 7% and 21.34% drop in the fertility of the females emerged from 4th and 5th instar nymphs respectively following the injection of the strongest dose (6.0 ug α-ecdysone per nymph). This reduction was 39.92% and 62.22% when same dose of 'Triol' was injected to the respective nymphs. As regards the topical treatment of this dose α-ecdysone caused a little further reduction (10.24% and 31.18%) in the fertility of the respective females as compared to those by injection. On the other hand,

the topical application of the same dose of 'Triol' to the larvae of the respective instars caused less fall in the fertility of the females (30.39% and 39.50%) as compared to those of injection.

The inhibitory effects of ecdysones and their analogues on the reproduction have been demonstrated in many other insects e.g., Musca domestica (Robbins et al. 1968; Thompson et al.,1971; Singh & Russell, 1980; Singh et al., 1982); Aedes aegypti (Robbins et al., 1970); Thompson et al., 1971), Blatella germanica (Robbins et al., 1968 & 1970; Thompson et al., 1971; Friedel et al., 1980), Manduca sexta (Robbins et al.,1968), Rhodnius prolixus (Mansingh, 1976), Dysdercus cingulatus (Jalaja et al., 1976), Stomoxys calcitrans (Wright & Kaplanis, 1970; Wright et al., 1971) and Anthonomous grandis (Earle et al., 1970). However, on the other hand, in certain insects e.g., Tenebrio molitor (Laverdure, 1969), Aedes aegypti (Spielman et al.,1971), Heteroneza pygmaea (Went, 1978) etc. these hormones have been found to stimulate ovarian development and oogenesis. The inhibitory effect of these steroids is generally related to vitellogenesis. There are two general processes involved in vitellogenesis. The first is the synthesis of vitellogenin by the fat body and its secretion into haemolymph, and the second is the uptake of vitellogenin from the haemolymph and its deposition into yolk granules in the oocytes (Doane, 1973). Ecdysones inhibit either the very synthesis of vitellogenin or

its uptake by the developing oocytes rendering them non-viable.

From these experiments on S. litura, D. obliqua and D. cingulatus it can be inferred that moulting hormones especially β-ecdysone and 'Triol' can be effectively used in controlling these insects. On S. litura larvae β-ecdysone has proved to be more toxic. But in all the three species 'Triol', an analogue of the ecdysone, has been found much successful in reducing adult emergence, fecundity and fertility. The fact that certain of these steroids block insect growth and development at concentration in the ppm to ppb range and inhibit ovarian development, indicates their potential as hormonal pesticides and chemosterilants. However, compounds with moulting hormone activity would be more expensive to produce because of their complex steroidal structures. Further, these steroids, unlike juvenile hormone, do not readily penetrate the insect cuticle and thus usually be taken up by ingestion. According to Robbins et al. (1970) these disadvantages, however, may eventually be overcome through the development of active compounds with simpler structures, microbiological synthesis and/or by obtaining these steroids from plant sources (phytoecdysones). The ecdysones and their analogues have a decided advantage over juvenile hormone compounds which selectively interfere with the terminal moult; the pupal-adult or nymphal-adult moult whereas ecdysones and their analogues block immature development

at any larval or pupal moult and thus could be fitted directly into our present insect control technology.

Fig. 8 Showing total mortality up to adult emergence following injection and ingestion of different doses of β-ecdysone and 'Triol' in the 5th and 6th instar larvae of Spodoptera litura.

(O———O) = β-ecdysone ..... 5th instar larvae

(●———●) = β-ecdysone ..... 6th instar larvae

(△———△) = 'Triol' ..... 5th instar larvae

(▲———▲) = 'Triol' ..... 6th instar larvae

(O-------O) = β-ecdysone ..... 5th instar larvae

(●-------●) = β-ecdysone ..... 6th instar larvae

(△-------△) = 'Triol' ..... 5th instar larvae

(△-------△) = 'Triol' ..... 6th instar larvae

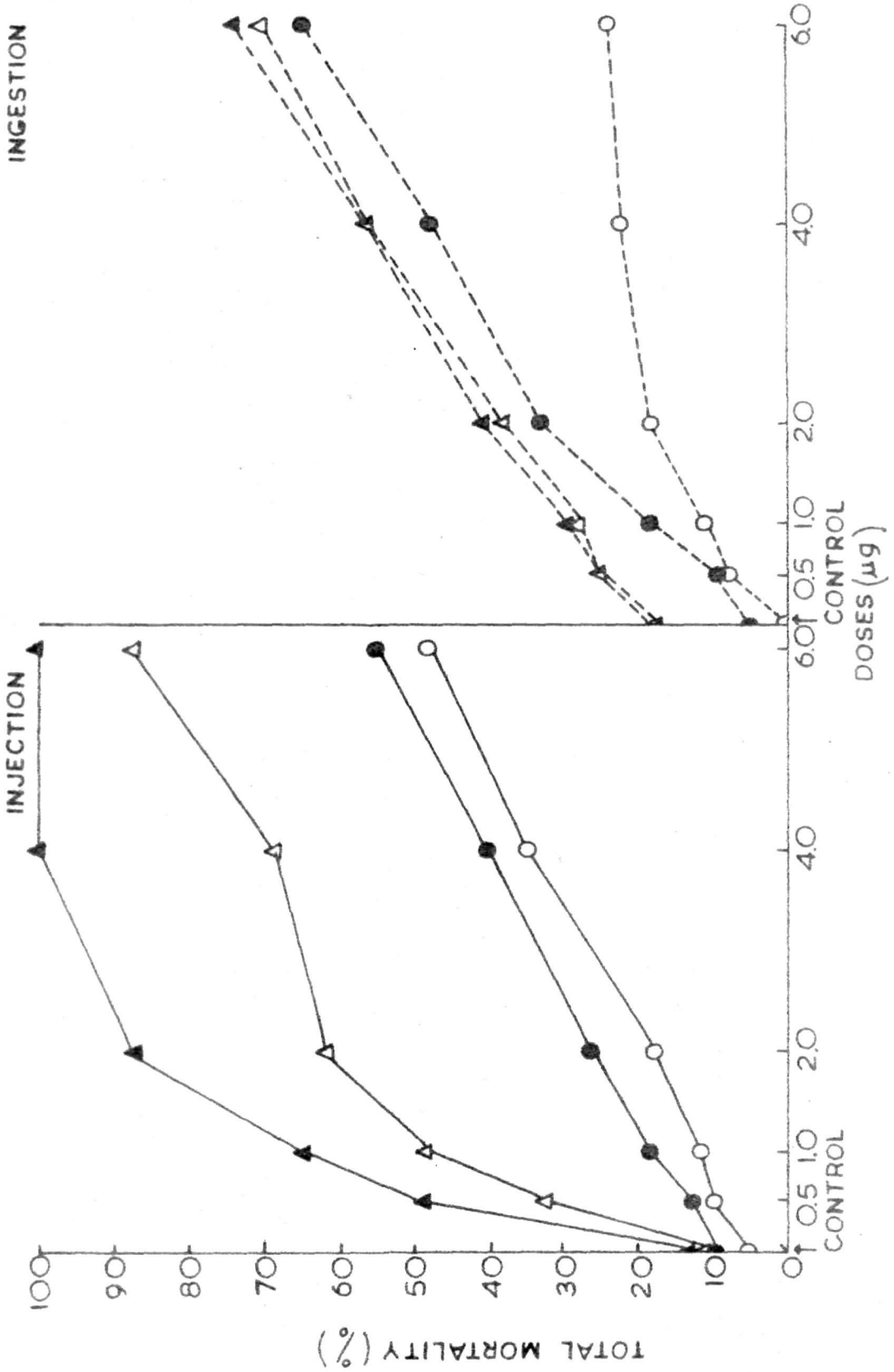
INGESTION
INJECTION
TOTAL MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
0.5 1.0 2.0 4.0 6.0
CONTROL
DOSES (µg)

FIG. 5

Fig. 6 Showing reduction in the fecundity of females emerged from 5th and 6th instar larvae of *Spodoptera litura* following injection and ingestion of different doses of β-ecdysone and 'Triol'.

(○———○) = β-ecdysone ..... 5th instar larvae

(●———●) = β-ecdysone ..... 6th instar larvae

(△———△) = 'Triol' ..... 5th instar larvae

(▲———▲) = 'Triol' ..... 6th instar larvae

(○-------○) = β-ecdysone ..... 5th instar larvae

(●-------●) = β-ecdysone ..... 6th instar larvae

(△-------△) = 'Triol' ..... 5th instar larvae

(▲------▲) = 'Triol' ..... 6th instar larvae

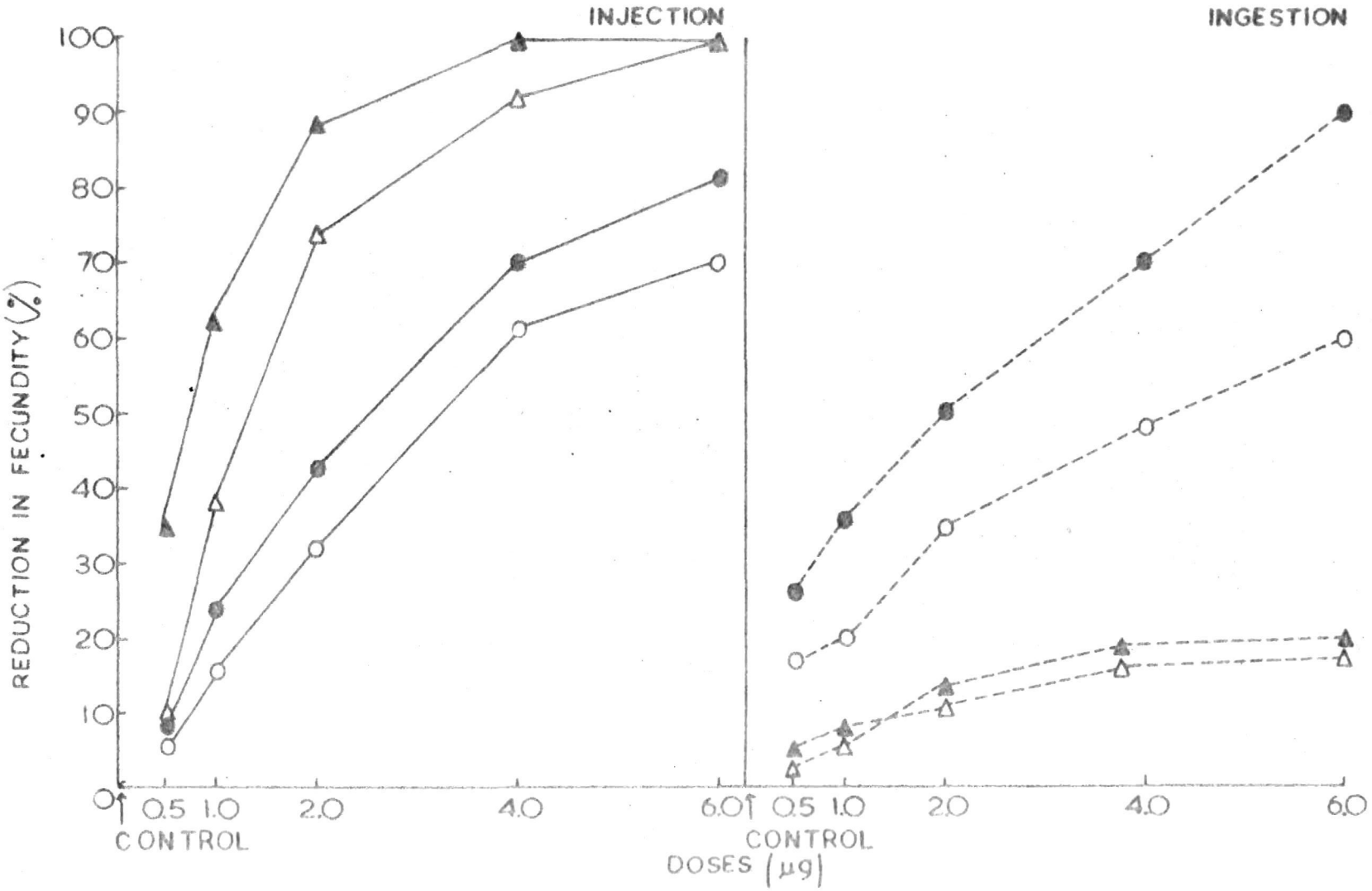
INJECTION
INGESTION
REDUCTION IN FECUNDITY (%)
100
90
80
70
60
50
40
30
20
10
0
0.5
1.0
2.0
4.0
6.0
CONTROL
DOSES (µg)

FIG. 6

Fig. 7 Showing reduction in the fertility of females emerged from 5th and 6th instar larvae of **Spodoptera litura** following the injection and ingestion of different doses of β-ecdysone and 'Triol'.

(○———○) = β-ecdysone ..... 5th instar larvae

(●———●) = β-ecdysone ..... 6th instar larvae

(△———△) = 'Triol' ..... 5th instar larvae

(▲———▲) = 'Triol' ..... 6th instar larvae

(○------○) = β-ecdysone ..... 5th instar larvae

(●------●) = β-ecdysone ..... 6th instar larvae

(△------△) = 'Triol' ..... 5th instar larvae

(▲------▲) = 'Triol' ..... 6th instar larvae

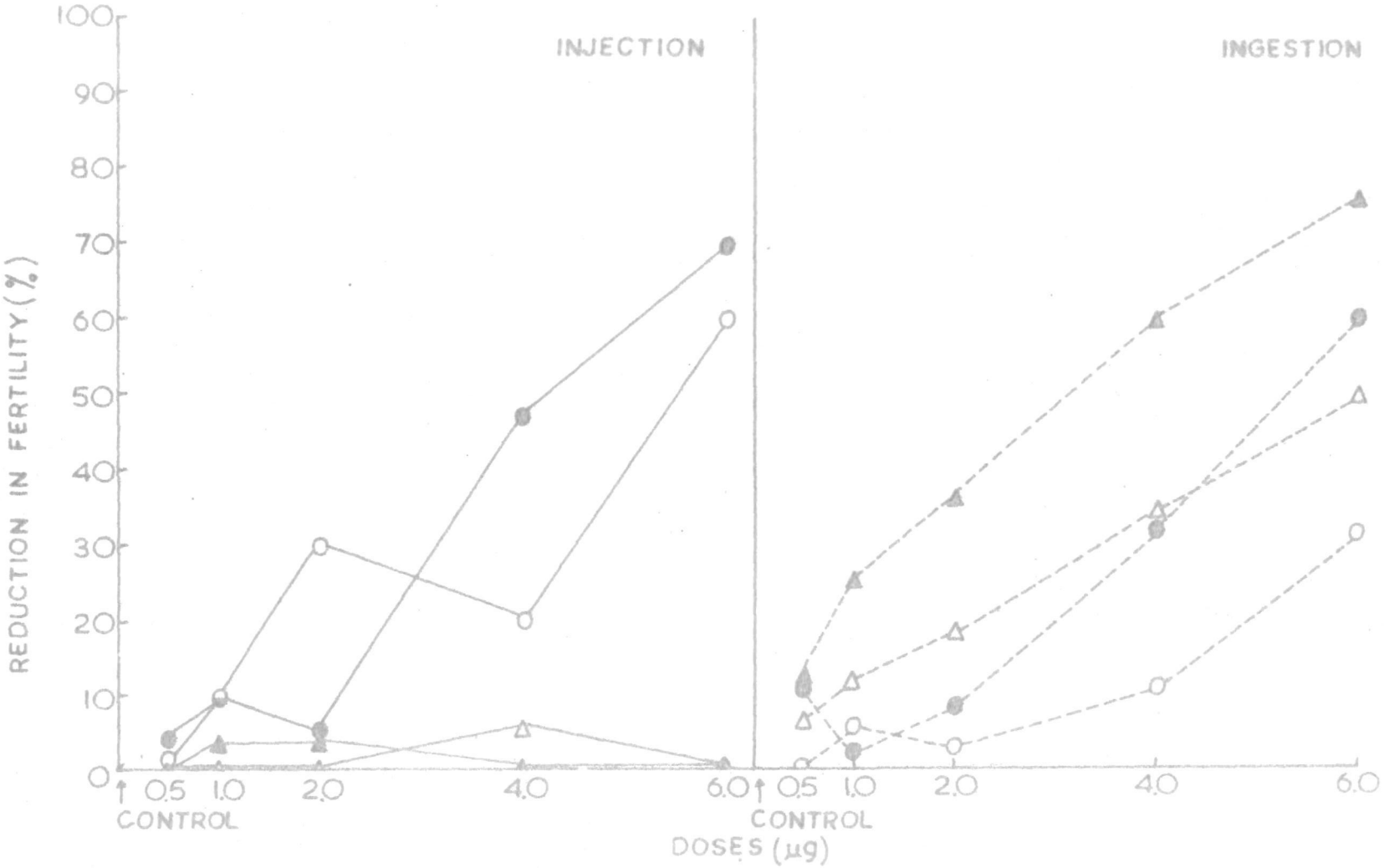
INJECTION
INGESTION
REDUCTION IN FERTILITY (%)
100
90
80
70
60
50
40
30
20
10
0
CONTROL
0.5
1.0
2.0
4.0
6.0
DOSES (µg)

FIG. 7

Fig. 8 Showing total mortality up to adult emergence following injection and ingestion of different doses of α-ecdysone and 'Triol' in the 5th and 6th instar larvae of Diacrisia obliqua.

(○———○) = α-ecdysone ..... 5th instar larvae

(●———●) = α-ecdysone ..... 6th instar larvae

(△———△) = 'Triol' ..... 5th instar larvae

(▲———▲) = 'Triol' ..... 6th instar larvae

(○-------○) = α-ecdysone ..... 5th instar larvae

(●-------●) = α-ecdysone ..... 6th instar larvae

(△-------△) = 'Triol' ..... 5th instar larvae

(▲-------▲) = 'Triol' ..... 6th instar larvae

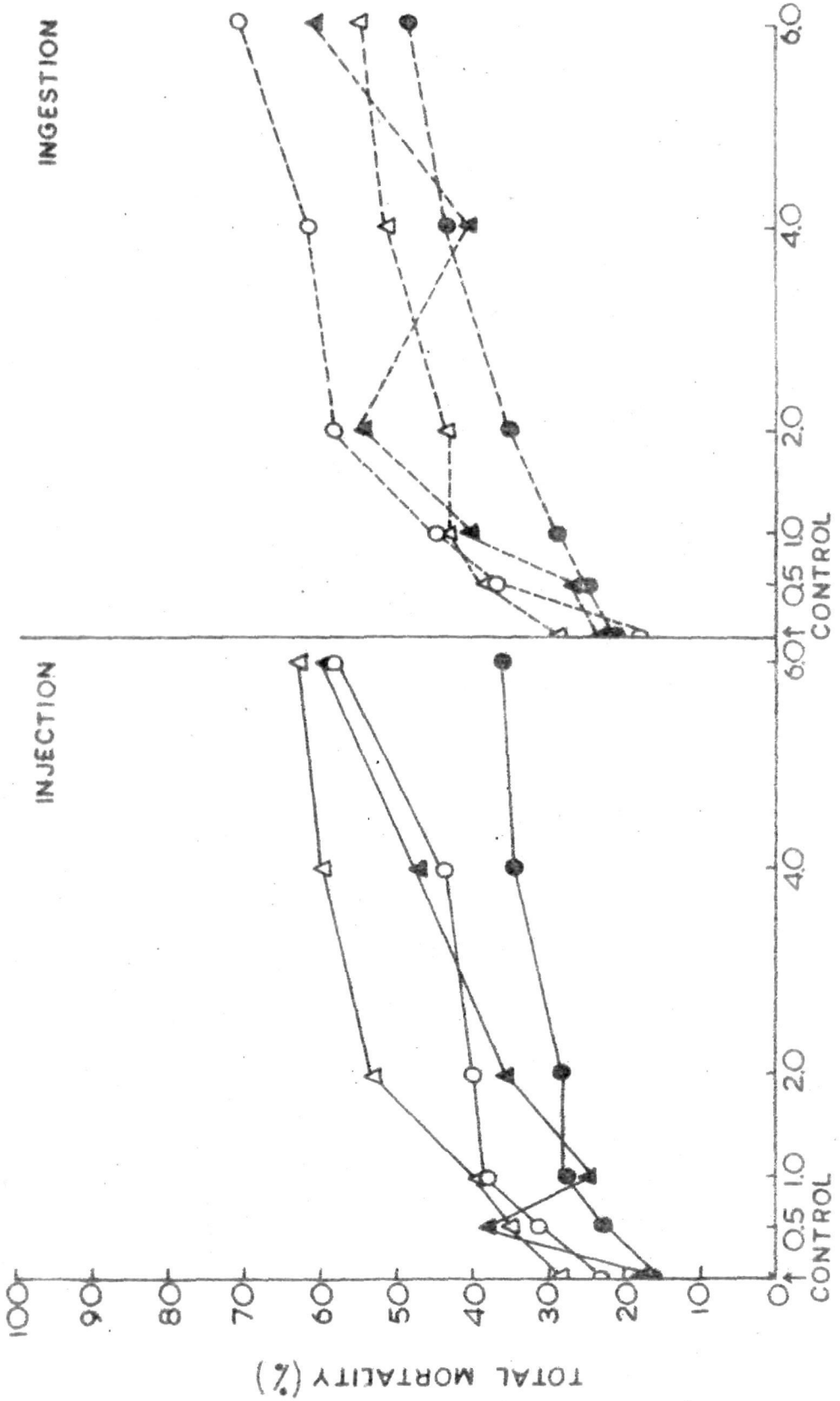
INGESTION
INJECTION
TOTAL MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
CONTROL
0.5
1.0
2.0
4.0
6.0
DOSES (µg)

FIG. 8

Fig. 9 Showing reduction in the fecundity of females emerged from 5th and 6th instar larvae of *Diacrisia obliqua* following the injection and ingestion of different doses of α-ecdysone and 'Triol'.

(○———○) = α-ecdysone ..... 5th instar larvae

(●———●) = α-ecdysone ..... 6th instar larvae

(△———△) = 'Triol' ..... 5th instar larvae

(▲———▲) = 'Triol' ..... 6th instar larvae

(○------○) = α-ecdysone ..... 5th instar larvae

(●------●) = α-ecdysone ..... 6th instar larvae

(△------△) = 'Triol' ..... 5th instar larvae

(▲------▲) = 'Triol' ..... 6th instar larvae

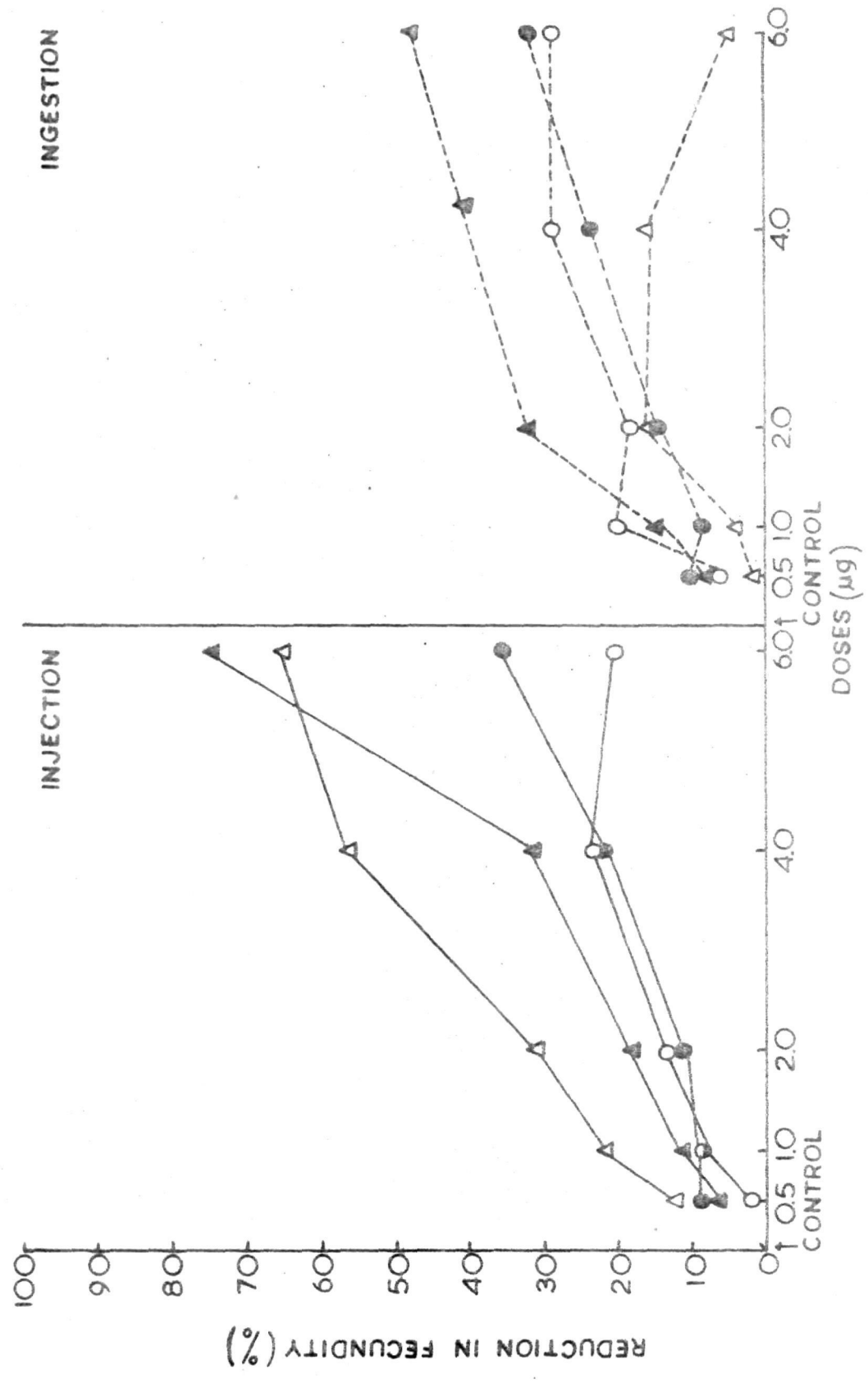
INJECTION
INGESTION
REDUCTION IN FECUNDITY (%)
100
90
80
70
60
50
40
30
20
10
0
CONTROL
0.5
1.0
2.0
4.0
6.0
DOSES (µg)

FIG. 9

Fig. 10 Showing reduction in the fertility of females emerged from 5th and 6th instar larvae of Diacrisia obliqua following the injection and ingestion of different doses of α-ecdysone and 'Triol'.

(○———○) = α-ecdysone ..... 5th instar larvae

(●———●) = α-ecdysone ..... 6th instar larvae

(△———△) = 'Triol' ..... 5th instar larvae

(▲———▲) = 'Triol' ..... 6th instar larvae

(○------○) = α-ecdysone ..... 5th instar larvae

(●------●) = α-ecdysone ..... 6th instar larvae

(△------△) = 'Triol' ..... 5th instar larvae

(▲------▲) = 'Triol' ..... 6th instar larvae

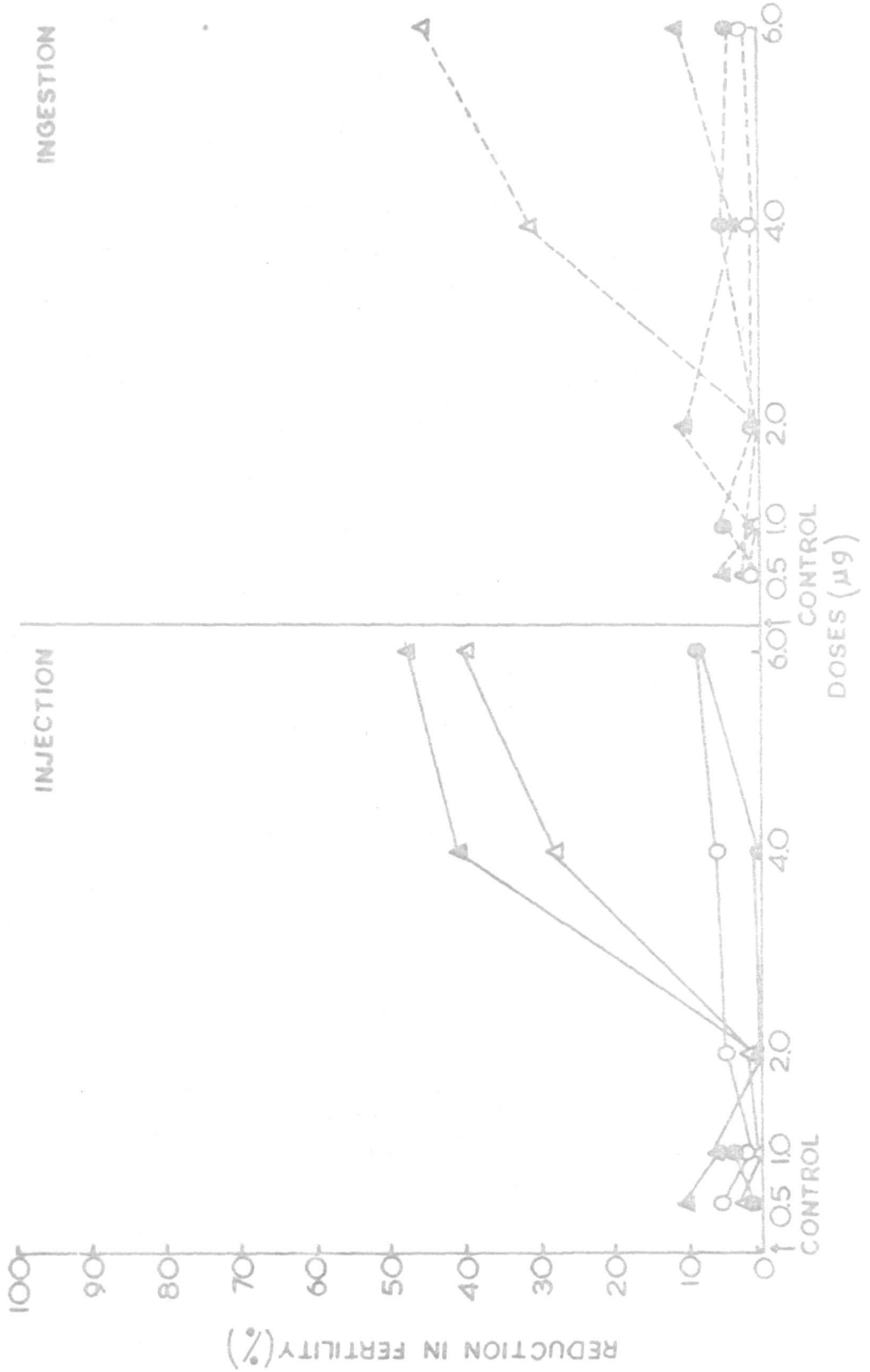
INGESTION
INJECTION
REDUCTION IN FERTILITY (%)
100
90
80
70
60
50
40
30
20
10
0
CONTROL
0.5
1.0
2.0
4.0
6.0
DOSES (µg)

FIG. 10

Fig. 11 Showing total mortality up to adult emergence following injection and topical treatment of different doses of α-ecdysone and 'Triol' to the 4th and 5th instar nymphs of *Dysdercus cingulatus*.

(○———○) = α-ecdysone ..... 4th instar nymphs

(●———●) = α-ecdysone ..... 5th instar nymphs

(△———△) = 'Triol' ..... 4th instar nymphs

(▲———▲) = 'Triol' ..... 5th instar nymphs

(○------○) = α-ecdysone ..... 4th instar nymphs

(●------●) = α-ecdysone ..... 5th instar nymphs

(△------△) = 'Triol' ..... 4th instar nymphs

(▲------▲) = 'Triol' ..... 5th instar nymphs

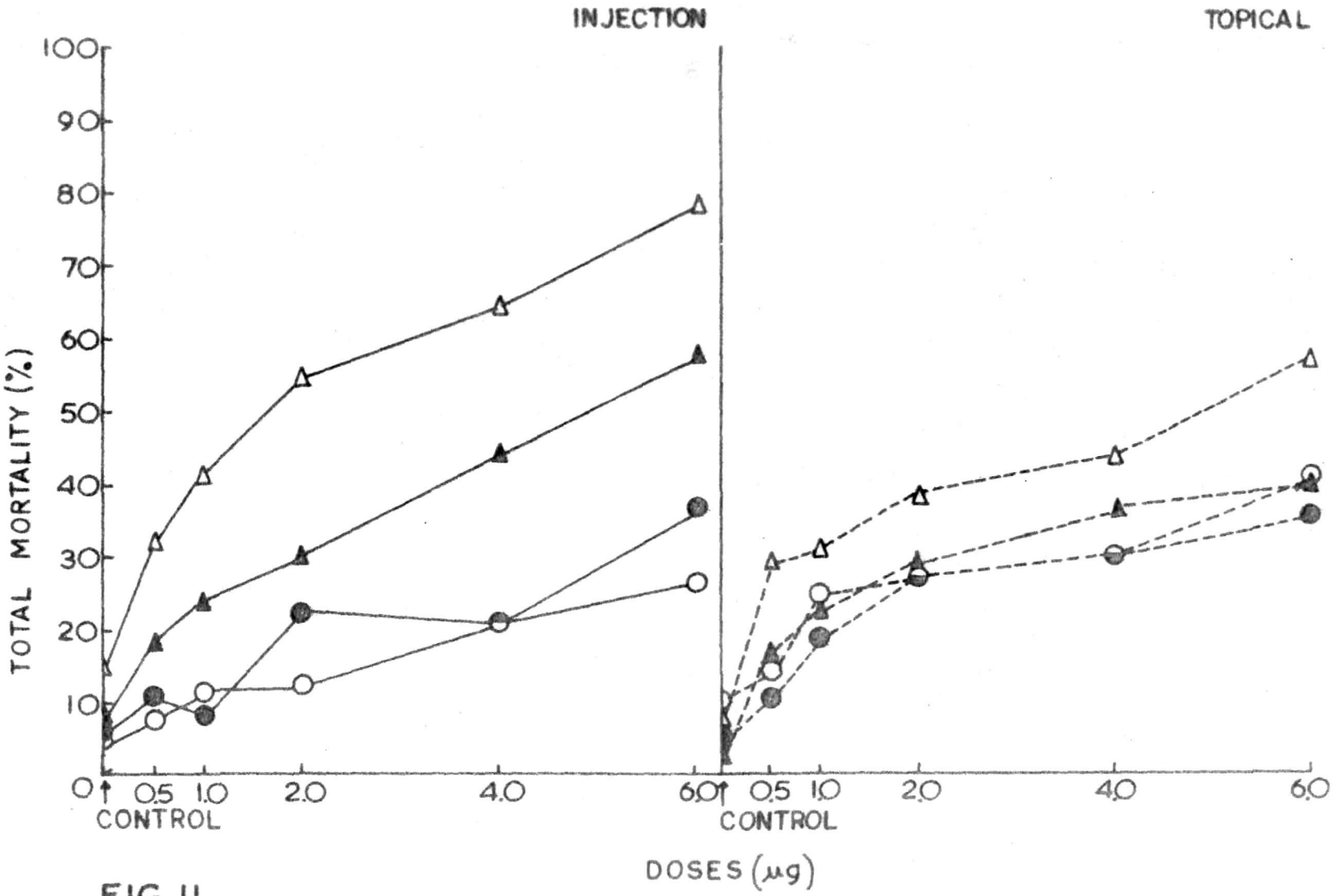
INJECTION
TOPICAL
TOTAL MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
0.5
1.0
2.0
4.0
6.0
CONTROL
CONTROL
DOSES (μg)

FIG. 11

Fig. 12 Showing reduction in the fecundity of females emerged from 4th and 5th instar nymphs of *Dysdercus cingulatus* injected or topically treated with different doses of α-ecdysone and 'Triol'.

(O———O) = α-ecdysone ..... 4th instar nymphs

(●———●) = α-ecdysone ..... 5th instar nymphs

(△———△) = 'Triol' ..... 4th instar nymphs

(▲———▲) = 'Triol' ..... 5th instar nymphs

(O-------O) = α-ecdysone ..... 4th instar nymphs

(●-------●) = α-ecdysone ..... 5th instar nymphs

(△-------△) = 'Triol' ..... 4th instar nymphs

(▲-------▲) = 'Triol' ..... 5th instar nymphs

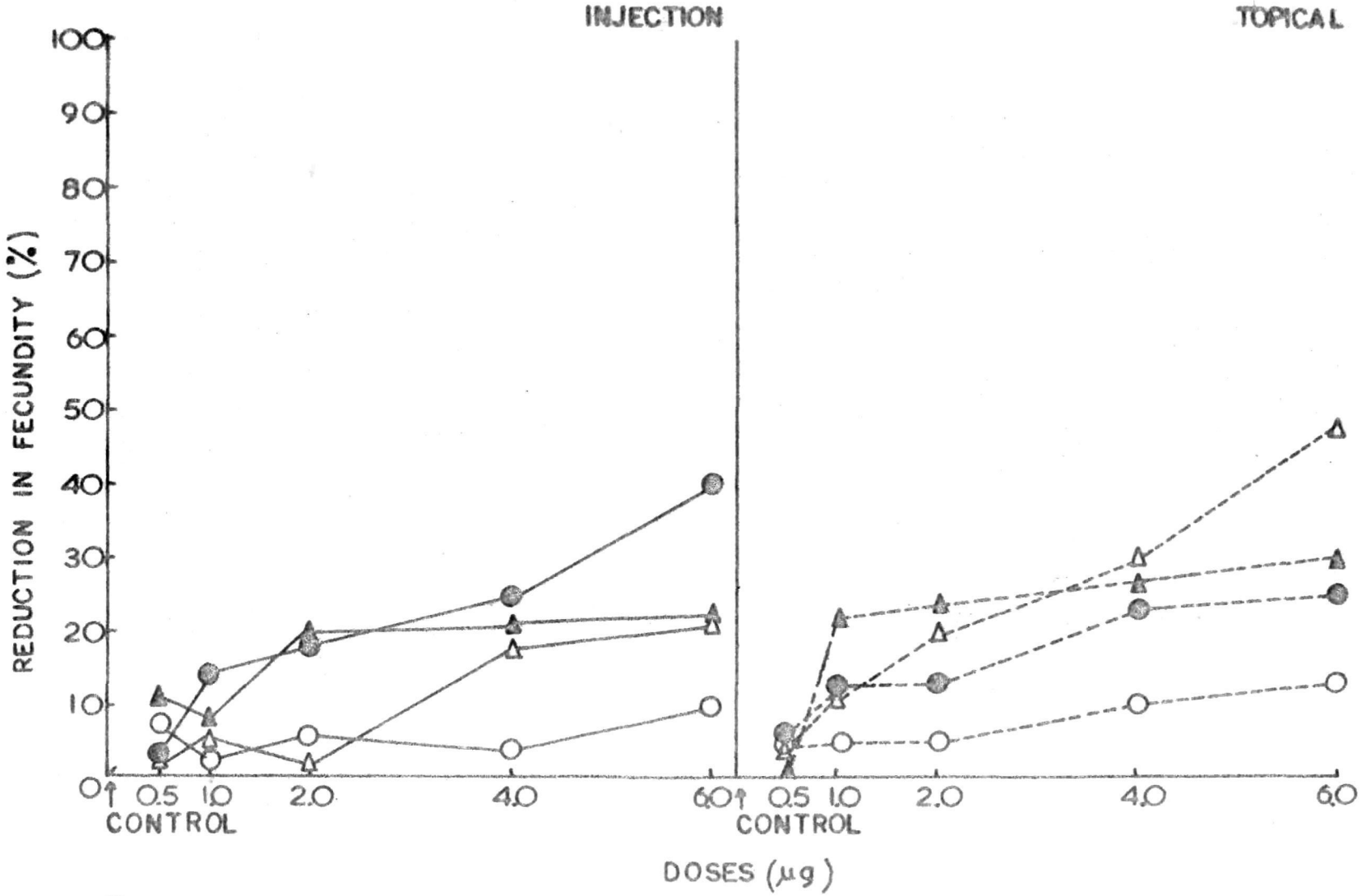
INJECTION
TOPICAL
REDUCTION IN FECUNDITY (%)
100
90
80
70
60
50
40
30
20
10
0
0.5
1.0
2.0
4.0
6.0
CONTROL
DOSES (µg)

FIG. 12

Fig. 13 Showing reduction in the fertility of females emerged from 4th and 5th instar nymphs of *Dysdercus cingulatus* injected or topically treated with different doses of α-ecdysone and 'Triol'.

(O———O) = α-ecdysone ..... 4th instar nymphs

(●———●) = α-ecdysone ..... 5th instar nymphs

(△———△) = 'Triol' ..... 4th instar nymphs

(▲———▲) = 'Triol' ..... 5th instar nymphs

(O------O) = α-ecdysone ..... 4th instar nymphs

(●------●) = α-ecdysone ..... 5th instar nymphs

(△------△) = 'Triol' ..... 4th instar nymphs

(▲------▲) = 'Triol' ..... 5th instar nymphs

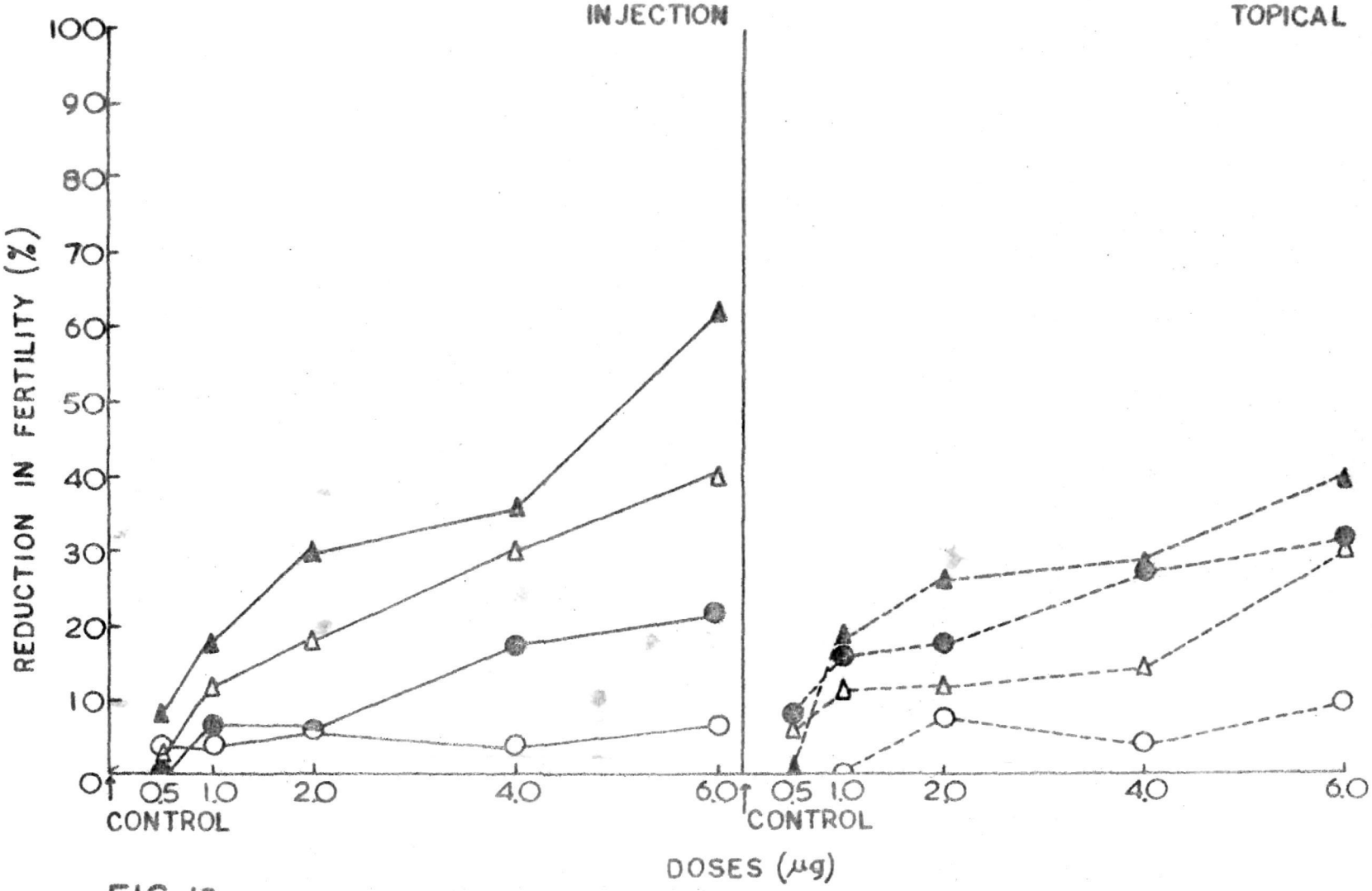
INJECTION
TOPICAL
REDUCTION IN FERTILITY (%)
100
90
80
70
60
50
40
30
20
10
0
0.5
1.0
2.0
4.0
6.0
CONTROL
0.5
1.0
2.0
4.0
6.0
CONTROL
DOSES (µg)

FIG. 13

SUMMARY

(1) The effect of moulting hormones such as α- and β-ecdysone as well as an analogue, 'Triol' was observed on the growth and reproduction of Spodoptera litura, Diacrisia obliqua and Dysdercus cingulatus.

(2) In S. litura, 5th and 6th instar larvae were individually injected or fed with β-ecdysone and 'Triol' in 0.5 ug, 1.0 ug, 2.0 ug, 4.0 ug and 6.0 ug dose per larva whereas in D. obliqua, the larvae of the respective instars were so treated with α-ecdysone and 'Triol' by the aforesaid dose. But in D. cingulatus the advanced stages of nymphs (4th and 5th instars) were treated with α-ecdysone and 'Triol' by injection or topical method of the doses as used for other two insects.

(3) In S. litura, following the injection of 0.5, 1.0, 2.0, 4.0 and 6.0 ug β-ecdysone/larva to either 5th or 6th instar larvae caused larval mortality which was relatively higher with stronger doses. Thus the injection of the strongest dose (6.0 ug) of β-ecdysone to the individual larva of 5th and 6th instars caused 42% and 45% larval mortality respec-

tively. Similar treatmwas seen after feeding the respective doses of this hormone and by the strongest dose the larval mortality was 34% and 60% respectively with regard to 5th and 6th instars.

(4) Although the injection of various doses of 'Triol' to the larvae of S. litura also resulted in larval mortality, that of the strongest dose (6.0 ug/larva) of 'Triol' caused 78% and 100% larval mortality respectively. However, mortality was 53% and 58% respectively when the same dose of this analogue was fed to the respective instar.

(5) Larval mortality was also found when α-ecdysone was injected or fed to the larvae of 5th or 6th instar of D. obliqua. Thus following the injection of 6.0 ug dose to the individual larva of the respective instars, larval mortality was 36% and 20%. The ingestion of same dose of this hormone caused 53% and 28% larval mortality respectively.

(6) The injection of 6.0 ug dose of the analogue 'Triol' to the 5th and 6th instar larvae of D. obliqua caused 37% and 43% larval mortality respectively whereas by feeding this dose of the analogue resulted in 26% and 38% larval mortality respectively.

(7) In D. cingulatus also nymphal mortality was found by all doses following the treatment of advanced nymphal stages with α-ecdysone and 'Triol'. The injection of 6.0 ug dose of α-ecdysone to the nymphs of 4th and 5th instars lead to 22% and 31% nymphal mortality respectively. The topical application of the same dose of this hormone caused 31% larval mortality in both the instars.

(8) Injection of 6.0 ug 'Triol' to the individual nymphs of the 4th and 5th instars of D. cingulatus lead to 64% and 49% nymphal mortality. But following the topical application of this dose the nymphs of either instar suffered 49% and 37% mortality.

(9) In all the three species, S. litura, D. obliqua and D. cingulatus, following the treatment with different doses by either method ofapplication the larval or nymphal mortality was relatively higher with stronger doses of all the ecdysones.

(10) The application of exogenous moulting hormones (α-, β- and 'Triol') also produced adverse effect on larval/nymphal longevity of S. litura, D. obliqua and D. cingulatus.

(11) In S. litura following the injection of any of the present doses of β-ecdysone to the 5th instar larvae

the longevity of these larvae remained unchanged. However, the longevity of 6th instar larvae was shortened by 20-24 hours following the injection of 6.0 ug β-ecdysone, but lower doses were ineffective in this regard.

(12) On the other hand when 6.0 ug -ecdysone was fed to the larvae of either 5th or 6th instar S. litura the longevity of both the instars was abbreviated and these were by 10-15 hours and 24-36 hours respectively. Further, by feeding also lower doses were usually ineffective.

(13) Application of the analogue, 'Triol', either by injection or feeding to the larvae of both 5th and 6th instars of S. litura abbreviated the longevity of either instar much more as compared to that of β-ecdysone treatment. Further both 4.0 ug and 6.0 ug 'Triol' was effective in this respect as compared to only 6.0 ug β-ecdysone.

(14) Unlike 5th instar larvae of S. litura, those of D. obliqua had reduction in their longevity following the injection of α-ecdysone (2.0, 4.0 and 6.0 ug doses) and when these doses were injected to 6th instar larvae, their longevity was unaffected.

(15) The longevity of both 5th and 6th instar larvae of D. obliqua remained unaffected following the ingestion of either dose of α-ecdysone.

(16) Injection of the corresponding doses of 'Triol' to the larvae of both the instars of D. obliqua did not affect their longevity. But the ingestion of higher doses of this analogue (4.0 and 6.0 ug dose) abbreviated the larval longevity of either instar by 24-32 hours and 36-40 hours (in 5th instar) and 18-22 and 22-26 hours (in 6th instar) respectively. Further, the abbreviation of the larval longevity was 30-40 hours in both the instars following the ingestion of 6.0 ug dose.

(17) In D. cingulatus, injection of α-ecdysone to 4th as well as 5th instar nymphs did not affect their nymphal longevity. In this respect this species differs from D. obliqua. It was only the injection 4.0 ug and 6.0 ug 'Triol' to the last stage nymphs (5th instar) which shortened their longevity by 16-24 hours and 20-28 hours respectively.

(18) Similar to the injection of α-ecdysone to the nymphs of D. cingulatus, its topical application also had no effect on the nymphal longevity of 4th instar of this species whereas such application on the 5th instar nymphs adversely affected

their longevity. However, topical application of all the present doses of 'Triol' affected the nymphal duration of both 4th and 5th instar nymphs.

(19) When 6th instar larvae of S. litura was injected or fed with 4.0 ug and 6.0 ug β-ecdysone it resulted in the occasional formation of supernumerary larval instars. By injection there was 16.66% supernumery larvae at 6.0 ug dose whereas by feeding the same dose 33.33% supernumerary larvae were formed but treatment with 'Triol' did not produce such effect. Further, formation of extra larval instar did not occur in either D. obliqua or in D. cingulatus following the treatment with exogenous moulting hormone.

(20) In D. obliqua, the treatment of the larvae by either α-ecdysone or 'Triol' produced malformed larvae represented as neotenic forms, precociously moulted forms, larvae with partial ecdysis and also larval-pupal intermediates. Such instances of larval malformation were prevalent more in case of the larvae treated with α-ecdysone either by injection or ingestion rather than in 'Triol' treated larvae.

(21) Malformation in case of S. litura and D. cingulatus

were completely absent following the application of any dose of β-ecdysone and 'Triol' in the former species and α-ecdysone and 'Triol' in the latter insect.

(22) Injection of β-ecdysone to either 5th or 6th instar larvae of S. litura showed occasional occurrence of malformed pupae but injection of 'Triol' did not produce malformed pupae. However, the ingestion of these hormone by either instar showed higher incidence of malformed pupae.

(23) Unlike S. litura, in D. obliqua, 6th instar larvae injected with either α-ecdysone or 'Triol' did not lead to the formation of abnormal pupae. But both the hormones when injected to 5th instar larvae resulted in the frequent formation of such pupae.

(24) Further, in D. obliqua, the ingestion of α-ecdysone by either 5th or 6th instar larvae did not produce malformed pupae. On the other hand, the ingestion of 4.0 ug and 6.0 ug doses of 'Triol' by either instar occasionally resulted in pupal malformation.

(25) In S. litura, following the application of all the present doses of both β-ecdysone and 'Triol' the number of adults produced from the treated larvae

was markedly reduced. In this respect injection of strongest dose (6.0 ug) of 'Triol' was most effective than that of β-ecdysone. It was evident by the fact that the number of adults emerging from 5th and 6th instar larvae injected with β-ecdysone was reduced by 42% and 45% respectively whereas the reduction was 78% and 100% when 'Triol' was injected to the respective instars.

(26) Although the ingestion of 6.0 ug β-ecdysone by the larvae of the respective instars did not show marked change in adult emergence (34% and 60% respectively) as compared to that of injection, following the feeding of same dose of 'Triol' reduction (53% and 58%) in adult emergence was less than that of injection of this dose of 'Triol'.

(27) The injection of highest dose of α-ecdysone (6.0 ug) to the 5th and 6th instar larvae of D. obliqua lowered the formation of adults by 36% and 20% respectively whereas following the injection of same dose of 'Triol' the reiuction was 37% and 43% respectively, showing that this ecdysone was not so active on this species as compared to S. litura in affecting adult formation.

(28) Following feeding of the strongest dose of α-ecdysone

to the larvae of D. obliqua the number of adults formed was reduced by 53% and 28% whereas that of 'Triol' reduced adult emergence by 26% and 38% respectively. In this respect feeding of 'Triol' on D. obliqua larvae was not as effective as it was on S. litura larvae.

(29) In D. cingulatus, adult emergence dropped by 22% and 31% respectively when 4th and 5th instar nymphs were injected with 6.0 ug/nymph of α-ecdysone whereas the injection of same dose of 'Triol' reduced adult number by 64% and 49% respectively showing that 'Triol' (analogue) was more active than α-ecdysone (natural ecdysone) in controlling adult emergence of this species.

(30) The topical application of strongest dose of α-ecdysone to both 4th and 5th instar nymphs did not appreciably change adult emergence as compared to injection of this dose whereas the topical application of the same dose of 'Triol' reduced percentage of adult emergence less than that of injection (49% and 37% respectively).

(31) There was no malformation in the emerged adults of S. litura and D. cingulatus following the applica-

tion of β-ecdysone and 'Triol' on the larvae of the first species and α-ecdysone and 'Triol' on the nymphs of the latter species. But in D. obliqua malformed adults emerged from the larvae treated with α-ecdysone as well as 'Triol'. Such adults had folded wings and maximum malformation (44.44%) appeared in the adults emerged from the 5th instar larvae injected with 6.0 ug.

(32) In S. litura, following the application of both β-ecdysone and 'Triol' to the advanced larval stages the fecundity of the females emerging from the larvae was effectively inhibited. There were 70.09% and 81.2% reductions in the number of eggs laid by the females emerged from the 5th and 6th instar larvae respectively, which were injected with strongest dose (6.0 ug) of β-ecdysone. However, the application of this dose of 'Triol' to the 5th instar larvae resulted in the development of mostly infecund females i.e. out of 6 females only one female laid 76 eggs whereas treated 6th instar larvae could not reach to adult stage.

(33) On the contrary, feeding of 6.0 ug β-ecdysone to the larvae of either instar suppressed the fecundity of their females by 60.54% and 89.95% which were higher than that of 'Triol' feeding (13.76% and 17.48%).

(34) In D. obliqua, 'Triol' (analogue) was usually more effective inhibitor of fecundity than that of α-ecdysone (natural ecdysone). The fecundity of the females emerged from the 5th and 6th instar larvae injected with 6.0 ug α-ecdysone was reduced by 20.24% and 34.59% respectively whereas that of 'Triol' decreased it by 65.01% and 75.88%.

(35) Following the ingestion of 6.0 ug α-ecdysone per larva of 5th and 6th instars of D. obliqua the fecundity of the emerged females decreased by 27.42% and 31.68% respectively whereas similar dose of 'Triol' dropped it respectively by 21.70% and 48.92%.

(3 6) In D. cingulatus, the fecundity of the females which emerged from the 4th and 5th instar nymphs injected with 6.0 ug ecdysone was respectively 9.99% and 39.10% less than that of the females developed from untreated nymphs (control). However, injection of similar dose of 'Triol' decreased the fecundity of the emerged females by 20.91% and 21.23% respectively. Thus on this species α-ecdysone was comparatively more effective than 'Triol' in reducing fecundity.

(37) Conversely, by topical application of 'Triol' the infecundity in the females was much higher than

that of α-ecdysone and maximum reduction was 47.32% by the topical treatment of 6.0 ug 'Triol' on 4th instar nymphs.

(38) Fertility of the eggs laid by the females emerged from the larvae of S. litura and D. obliqua and nymphs of D. cingulatus which were treated with the respective ecdysones was also reduced.

(39) In S. litura following the injection of 6.0 ug β-ecdysone per larva there was 60.6% and 70.15% drop in the fertility of the eggs of the females emerged from the 5th and 6th instar larvae. The ingestion of the same dose of 'Triol' (6.0 ug) to the 5th instar larvae lead to high infecundity and out of 76 eggs, laid by only one female out of 6 females, 45 eggs were viable. However, following the injection of 6.0 ug 'Triol' to the 6th instar larvae there was 100% larval mortality so the fertility of the female that would have emerged from these larvae could not be assessed.

(40) Ingestion of the same dose of either β-ecdysone or 'Triol' to the larvae of the respective instars of S. litura produced only 32.92% and 60.33% fertile eggs respectively (by β-ecdysone) and 80.58% and 78.76% respectively (by 'Triol').

(41) The fertility of the eggs laid by D. cingulatus females was also adversely affected by the application of both α-ecdysone and 'Triol'. The fertility of the females emerged from 5th and 6th instar larvae injected with 6.0 ug α-ecdysone was reduced by 8.26% and 8.60% respectively. The same dose of 'Triol' caused 40.77% and 47.48% reduction in the fertility.

(42) There was 2.27% reduction in the fertility of the females which emerged from either 5th or 6th instar larvae of D. obliqua fed with 6.0 ug dose of α-ecdysone. On the other hand, feeding the same dose of 'Triol' reduction was 47.25% and 12.51% respectively.

(43) In D. cingulatus 'Triol' was more effective than α-ecdysone when applied either by injection or topically. There was 7% and 21.24% drop in the fertility of the females emerged from 4th and 5th instar nymphs respectively following the injection of the strongest dose (6.0 ug α-ecdysone/nymph). This reduction was 39.92% and 62.22% when same dose of 'Triol' was injected to the respective nymphs. Topical treatment of this dose of α-ecdysone caused a little further reduction (10.24% and 31.18%).

On the other hand, the opical application of the same dose of 'Triol' to the larvae of the respective instars caused less fall in the fertility of females (30,29% and 39,50%).

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